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TABLE OF CONTENTS

A method for measuring resistance to defoliation diseases in tomato and other Lycopersicon species ..... SETH BARTON LOCKE 937

Control of cotton wilt and nematodes with a soil fumigant. A. L. SMITH 943

A simplified method of purifying tomato bushy-stunt virus for electron microscopy. RUSSELL L. STEERE AND ROBLEY C. WILLIAMS 948

Chemical treatment of soybean seed in relation to nodulation by nodule bacteria ..... M. F. KERNKAMP 955

Bacteria in the storage organs of healthy plants. IAN W. TERVET AND JOHN P. HOLLIS 960

Microascus trigonosporus from cereal and legume seeds. M. D. WHITEHEAD, M. J. THIRUMALACHAR, AND J. G. DICKSON 968

The effect of soaking cotton seed on the incidence of angular leaf spot in New Mexico and Arizona. A. R. LEDING AND L. A. BRINKERHOFF 974

An evaluation of certain substituted phenol esters for the treatment of cotton seed ..... C. H. ARNDT 978

Strains of Mycosphaerella fragariae ..... A. G. PLAKIDAS 988

The movement of crown-gall bacteria in isolated stem fragments of sunflowers ..... R. S. DE ROPP 993

Some studies of curly top of flax ..... N. J. GIDDINGS 999

Wheats immune from soil-borne mosaic viruses in the field, susceptible when inoculated manually ..... H. H. MCKINNEY 1003

Dip and gas treatments for the reduction of post-harvest decay in Texas lemons ..... A. LLOYD RYALL AND G. H. GODFREY 1014

A comparison of fungicidal treatments for the control of decay in California cantaloupes. W. R. BARGER, JAMES S. WIANT, W. T. PENTZER, A. LLOYD RYALL, AND D. H. DEWEY 1019

Phytopathological Notes 1025

Bird's eye spot disease of Hevea rubber in Nicaragua. ARTHUR G. KEVORKIAN

Powdery mildew resistance in cucumber ..... PAUL G. SMITH

"Needle curl" of shortleaf pine seedlings ..... L. W. R. JACKSON

The occurrence of Phytophthora parasitica on corn. ARNOLD J. ULLSTRUP AND C. M. TUCKER

A simple method of preserving tobacco-mosaic sera. T. MATSUMOTO AND CHIN-HOW LEE

A seedling blight of avocado caused by Phytophthora palmivora ..... ROBERT A. CONOVER

Sample size and plot size for testing resistance of strawberry varieties to Verticillium wilt. RICHARD E. BAKER AND VICTOR VOTH

Effect of 2,4-D in culture medium on the growth of three pathogenic fungi ..... WAYNE M. BEVER AND F. W. SLIFE

Index to Volume 38

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A METHOD FOR MEASURING RESISTANCE TO DEFOLIATION DISEASES IN TOMATO AND OTHER LYCOPERSICON SPECIES[1]

SETH BARTON LOCKE[2,3]

(Accepted for publication May 31, 1948)

INTRODUCTION

Two defoliation diseases of tomato, namely, early blight caused by *Alternaria solani* (A. and M.) Jones and Grout, and septoria leaf spot, caused by *Septoria lycopersici* Speg., are commonly destructive east of the Rocky mountains. Early in 1939, a project was undertaken at the Arkansas Agricultural Experiment Station which had for its goal the development of horticulturally superior strains of tomato possessing resistance to these two diseases. As the first step toward this goal it was necessary to devise a technique for accurately measuring resistance to the fungi in question. Various procedures have been used for this and similar purposes in the past.

The literature contains numerous reports (1, 2, 5, 7, 9, 12, 13, 17, 19, 20) which compare tomato varieties exposed under field conditions to natural infection by the organisms named. These tests have not been entirely satisfactory because the principal environmental factors influencing infection and symptom expression were uncontrolled, and because several pathogens often attacked the plants simultaneously. Artificial inoculation with pure cultures of the pathogens under partially controlled conditions in the greenhouse or inoculation chambers (1, 3, 8, 10, 15) has overcome some of the difficulties which are inherent in the field test. The procedure described here is of the latter type, and its distinctive features are (a) the use of detached leaf material for inoculation, (b) inoculation with pure cultures of the pathogens, and (c) evaluation of host reaction on the basis of lesion diameter. A preliminary report (11) on this procedure was made in December, 1941.

Extensive use of detached leaves has been made by others in studies involving obligate parasites and to a lesser extent with facultative parasites. A review of the literature on detached leaf culture including application to studies on susceptibility of hosts and virulence of pathogens has been published recently (21). Detached tomato leaflets were used by Douglas (6) to compare the response of tomato varieties to inoculation with an undetermined species of *Alternaria*. Bonde used a similar method to compare strains of *A. solani* with respect to virulence on potato. More recently

[1] Research Paper 862, Journal Series, University of Arkansas. Published with the permission of the Director of the Arkansas Agricultural Experiment Station.

[2] Formerly Assistant Plant Pathologist, Arkansas Agricultural Experiment Station, now Associate Plant Pathologist, Washington State College.

[3] The author is indebted to Dr. V. H. Young and other colleagues of the Department of Plant Pathology, University of Arkansas, and to Dr. W. H. Tharp and John C. Dunegan of the Bureau of Plant Industry for advice and criticism offered during the investigation reported here and in the preparation of the manuscript; and to Dr. C. B. Wadleigh of the U. S. Salinity Laboratory, Riverside, California, for advice on statistical treatment of the data.

Wellman (18) reported that the use of excised tomato leaflets for comparing virulence of strains of *A. solani* on tomato was unsuccessful because of rapid breakdown of the tissues caused by secondary organisms.

PROCEDURE

Plant Material

Plant material was selected for uniformity in age and absence of disease and insect injuries. The best results were obtained when young, vigorous plants, 8 to 10 inches tall, were used, but satisfactory results were also obtained with material from older plants, provided the plants were growing vigorously. The leaflets were taken from near the top of the plants, preference being given to leaves that were fully expanded but which still showed no signs of senescence. Usually the three apical leaflets of a leaf were taken but occasionally, where the leaflets were small, it was necessary to take five leaflets in order to provide space for the necessary number of inoculations. These were placed immediately in individual moist chambers consisting of 10-cm. Petri dishes whose covers were lined with two layers of moistened filter paper. Care was taken to avoid wilting because wilted leaflets did not regain turgidity after being placed in the moist chambers.

Cultures

The pathogens were originally isolated from naturally infected tomato leaves by transferring single spores to potato-dextrose-agar plates, where the early stages of growth were observed under low magnification in order to make certain that no contaminants were present. Cultures of *Septoria lycopersici* appeared to be fairly stable with regard to cultural characteristics and virulence. *Alternaria solani*, when freshly isolated, on potato-dextrose agar, produced the wine-red pigment described in the literature as characteristic of the species (4, 14, 16), but later gave rise to non-pigmented sectors. Only pigmented cultures were used for inoculum. Fresh isolations were made from naturally infected tomato foliage each season, and transfers were made only from pigmented sectors of the parent cultures. It has been shown that differences in virulence exist among different isolants of *A. solani* (18). However, this could not be a source of error in these tests because identical inocula were used for all comparisons between strains of the host plant.

Alternaria solani was cultured on potato-dextrose-agar plates, a single planting being made at the center of the plate. Greatest uniformity of growth was obtained when the medium was rather deep (5 mm.). The cultures were kept at room temperature in diffused light until the growth had nearly covered the plate, when the cultures were ready for use in the preparation of inoculum. *Septoria lycopersici* pycnospores were planted thickly over the entire surface of potato-dextrose-agar slants. Numerous, competing colonies developed and gave rise to an abundance of pycnidia and pyc-

nospores after from seven to eight days at room temperature in diffused light.

Preparation of Inoculum

Inoculum consisted of a suspension of pycnospores in the case of *Septoria lycopersici* and of mycelial fragments in the case of *Alternaria solani*.[4] The *A. solani* inoculum was prepared by macerating an entire plate culture in 50 cc. of sterile soil extract[5] in a mechanical blender (3). The *S. lycopersici* inoculum was prepared by flooding the surface of a slant culture with 10 cc. of sterile soil extract and agitating for a few minutes by rolling the tube between the hands. The resultant spore suspension was poured off and made up to 50 cc. with additional soil extract.

Inoculation and Incubation

Inoculation with mycelial or spore suspensions was accomplished by placing a droplet of the suspension on the lower surface of the tomato leaflet using a 3-mm. loop. A needle puncture was then made in the leaf tissue directly beneath the drop of inoculum. Inoculation without wounding often resulted in delayed infection, infection at several points around the periphery of the inoculum droplet, or in no infection. Wounding insured immediate and localized infection. The test is thus a measure of resistance to invasion as distinct from resistance to entry.

Ten inoculations were made on the leaf material in each moist chamber. Each plant was represented by the leaf material in one moist chamber in each of five series of inoculations. Thus, there was a total of fifty inoculations for each plant included in the test. Inoculation of any one series was completed on the same day that it was started, but different series were frequently inoculated on different days.

Inoculated leaflets were incubated at room temperature in diffused sunlight, for three days in the case of *Alternaria solani*, and for eight days in the case of *Septoria lycopersici*. Attempts to incubate the leaves in darkened chambers under controlled temperature resulted in the leaves becoming etiolated and subject to rotting by other organisms.

Evaluation of Host Response

Evaluation of resistance or susceptibility was based upon the relative sizes of the lesions resulting from inoculation. Each lesion was compared with a graded series of circular spots having known diameters and arranged for convenience along the edge of a card. The diameter of the circular spot corresponding most closely in area to the lesion was recorded.

[4] In some of the early tests small, 2×2 mm., blocks were cut from the plate culture of *A. solani*, and these were used to inoculate the leaflets by placing them fungus-side-down on the lower surface of the leaflets. This procedure was later abandoned in favor of the less tedious use of the mycelial fragment suspension.

[5] The soil extract was prepared by shaking 500 gm. greenhouse compost with 1000 cc. distilled water, allowing the soil to settle, decanting the clear extract, and making up to 1000 cc. with distilled water. Spores of *A. solani* and *S. lycopersici* germinated better in soil extract than in tap or distilled water.

In making comparisons the lesion was viewed from the upper surface of the leaflet, that is, from the side opposite the one on which the leaf was inoculated. In many cases the lesions were not circular, but it was nevertheless a simple matter to select a standard spot having nearly the same area. Occasionally the leaflets became watersoaked beyond the area occupied by the lesion. By holding the leaflet before a strong light, the approximate limits of the lesion could be determined.

The basic data consisted of the averages of the diameters of the ten lesions in each of the moist chambers. Thus, there were five such mean lesion diameters for each plant in the test, one in each of five series. These were subjected to an analysis of variance in which the variabilities associated with series and with strains of plant material were segregated. The residual variability was used to estimate experimental error. This experimental design is similar to the completely randomized block design commonly used in field experiments, the different inoculation series corresponding to the blocks in the field experiment.

TABLE 1.—*Efficiency of the detached-leaf method for measuring resistance to Alternaria solani and Septoria lycopersici in Lycopersicon species*

Pathogen and test	Material tested	Strains	Mean lesion diameter		
	Species		LDRS[b]	D[c]	D/LDRS[d]
Alternaria solani		*number*	*mm.*	*mm.*	
2	*L.*[a] *esculentum*	9	2.00	2.74	1.37
4	do	11	1.68	1.88	1.12
2	*L. glandulosum*	5	2.02	2.06	1.02
2	*L. hirsutum*	7	2.37	4.34	1.83
4	do	8	2.59	3.60	1.39
2	*L. peruvianum*	8	1.28	2.40	1.88
4	do	12	2.15	2.16	1.00
2	*L. pimpinellifolium*	22	1.66	2.34	1.41
4	do	20	1.81	1.86	1.03
5	Tomato varieties	15	2.38	3.64	1.53
6	do	15	4.43	5.46	1.23
7	do	15	0.91	1.32	1.45
8	do	17	1.91	1.94	1.02
Septoria lycopersici					
1	*L. esculentum*	8	0.96	1.42	1.48
3	do	9	1.32	1.18	0.89
1	*L. glandulosum*	5	0.74	1.02	1.38
1	*L. hirsutum*	7	0.36	1.72	4.78
3	do	7	0.29	0.40	1.38
1	*L. peruvianum*	11	0.98	1.24	1.27
3	do	11	1.96	1.58	0.81
1	*L. pimpinellifolium*	16	1.16	1.92	1.66
3	do	18	1.31	2.18	1.66
1	Tomato varieties	6	1.13	0.82	0.73
3	do	6	1.47	1.78	1.21

[a] *Lycopersicon.*

[b] Least difference required for significance at odds of 19:1.

[c] Greatest difference observed.

[d] Ratio of greatest observed difference to least difference required for significance at odds of 19:1.

DISCUSSION

The procedure described has a number of advantages over field tests and many of the greenhouse and laboratory tests previously employed. Some of these are as follows:

1. It provides for control of the important environmental factors which may influence the virulence of the pathogen and the reaction of the host.

2. It enables the testing of host reaction to a single pathogen or a single strain of the pathogen without the confusion resulting from the activities of additional pathogens as is often the case with field tests. Furthermore, it is possible to test individual host plants independently for reaction to two or more different pathogens. Very susceptible plants are not eliminated and are available for genetic studies or testing against other pathogens.

3. The test is independent of such factors as growth habit and fruit load which affect the reactions of plants in field tests.

4. The test measures physiologic resistance to invasion irrespective of resistance to entry, and can be altered easily to give a measure of the latter.

5. The use of lesion diameter as a measure of host reaction reduces the personal factor to a minimum and permits statistical treatment of the data.

In the course of four years, this test has been applied to a wide range of plant material, including commercial tomato strains, other *Lycopersicon* species, and a *Lycopersicon* species-hybrid as well as some of its backcrossed and selfed progenies. Its use has facilitated the discovery of a source of practical resistance to both of the diseases in question, and it has aided progress toward the goal of incorporating genetic factors controlling such resistance into horticulturally superior tomato strains. Reference to table 1 will give the reader an indication of the magnitude of the differences in mean lesion diameter obtained in representative tests and how they compare with the least differences required for significance.

SUMMARY

A laboratory test was devised for measuring the relative resistance or susceptibility of tomato strains and *Lycopersicon* species to two commonly destructive defoliation diseases, namely, early blight (*Alternaria solani*) and septoria leaf spot (*Septoria lycopersici*).

The test makes use of artificial inoculation with pure cultures of the pathogens into detached leaflets which are then incubated in moist chambers. Evaluation of resistance or susceptibility is based upon lesion diameter attained during a uniform period of incubation.

The test has proven sufficiently sensitive to show small but significant differences in susceptibility to these diseases among commercial tomato strains. It has been useful in the discovery of a source of practical resistance to early blight and septoria leaf spot and has aided in progress

toward incorporating genetic factors controlling this resistance into horticulturally superior tomato strains.

UNIVERSITY OF ARKANSAS,
FAYETTEVILLE, ARKANSAS.

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CONTROL OF COTTON WILT AND NEMATODES WITH A SOIL FUMIGANT[1]

A. L. SMITH[2]

(Accepted for publication June 10, 1948)

INTRODUCTION

Cotton wilt caused by *Fusarium oxysporum* f. *vasinfectum* (Atk.) Snyder and Hansen, which occurs widely in southeastern and south central United States, is usually controlled by planting resistant varieties. The root-knot nematode, *Heterodera marioni* (Cornu) Goodey, is commonly associated with cotton wilt in light soils. It is generally recognized that cotton wilt tends to increase in the presence of root-knot nematode. Taylor and associates (5, 6) and Smith (2) have mentioned the meadow nematode, *Pratylenchus pratensis* de Man, as an important factor in increasing wilt and reducing yields. Subsequent observations by the writer suggest that the meadow nematode is of considerable importance in fields where wilt becomes severe and where resistant varieties become wilted and stunted and do not produce satisfactorily. The present study is concerned with attempts to control this disease complex with a soil fumigant. Dowfume W-10[3] was used in all studies reported here.

LITERATURE REVIEW

The possibility of controlling the wilt-nematode complex was demonstrated by Taylor and associates (5, 6). In their studies made at Lumberton, North Carolina, several varieties were compared on soil treated with carbon-disulphide and on non-treated soil. They found that the carbon-disulphide treatment controlled nematodes sufficiently to enable various wilt-resistant and wilt-tolerant varieties of cotton to become established and grow satisfactorily. Only a moderate amount of wilt developed in treated plots. A parallel situation was reported by Young (7) with tomato wilt. The application of 300–600 lb. chloropicrin per acre delayed the appearance of wilt symptoms 20–40 days and reduced the wilted plants to 7–10 per cent in the treated plots, while there were from 72 to 100 per cent wilted plants in non-treated plots. Godfrey (1) and several later investigators have demonstrated the fungicidal properties of chloropicrin. The fungicidal properties of ethylene dibromide used in the present study

[1] Cooperative investigations by the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture and the Alabama and Georgia Agricultural Experiment Stations. Published with the approval of the Directors of the Alabama and Georgia Agricultural Experiment Stations.

[2] Pathologist, Division of Cotton and Other Fiber Crops and Diseases. The author acknowledges the assistance of Fred Schultz of the Alabama Station Staff, in conducting the field tests at Tallassee, Alabama.

[3] Dowfume W-10 contains approximately 10 per cent ethylene dibromide and 90 per cent naphtha B. This material was furnished by the Dow Chemical Company, Midland, Michigan.

have not been thoroughly investigated, although Stark and associates (4) found ethylene dibromide ineffective in controlling damping-off of pea seedlings in greenhouse studies.

FUMIGATION OF MODERATELY INFESTED SOIL

In 1946, a test was run at Plains, Georgia. The soil was a Greenville sandy loam of low productivity with a moderate wilt, root-knot, and meadow nematode infestation. The fumigant was applied April 4 at the rate of 31 gallons per acre. The test consisted of 4 replications with rows 70 feet long. The rows were 42 inches wide in all tests reported here. The fumigant was injected to a depth of 6 inches at points 18 inches apart. One line of injection points was placed directly in the row to be planted and these were staggered with a second line of points between the planted rows. A non-treated buffer row was included between treated and non-treated plots. Coker 4 in 1–7, a wilt-resistant and root-knot tolerant variety, was compared with Deltapine 14, a variety susceptible to both wilt and root knot. Treated plots alternated with non-treated control plots.

The plantings were made April 9 under favorable conditions. A slight phytocidal action of the Dowfume W-10 was apparent by the reduced number of seedlings. The differences in emergence on treated and non-treated plots were not great. A satisfactory stand of Deltapine and a poor stand of Coker 4 in 1–7 were obtained. Wilt data were recorded by removing dead plants at intervals and by counting the remaining wilted plants and non-wilted plants at the end of the season. After picking the cotton, the roots were examined and root-knot indices determined according to the method described by Smith and Taylor (3). The results are shown in table 1.

TABLE 1.—*The effect of soil treatment with Dowfume W-10 on the yield, percentage of wilt, and the root-knot index of cotton varieties grown at Plains, Georgia, in 1946 and at Tallassee, Alabama, in 1947*

Location and variety	Treatment rate (Gal. per acre)	Lint yield per acre (Lb.)		Percentage increase in yield from treatment	Wilted and dead plants (Per cent)		Root-knot index	
		Con.[a]	Treated		Con.	Treated	Con.	Treated
Plains, Ga.								
Coker 4 in 1–7	31	197	281	42	2.6	0.5	50.2	2.7
Deltapine 14	31	162	336	108	21.3	0.9	96.8	6.9
Tallassee, Ala.								
Coker 4 in 1–7	37.5	367	1067	191	52.7	1.8	95.7	12.6
	12.5	495	963	95	37.7	6.4	91.1	57.8
Deltapine 14	37.5	64	904	1312	96.8	3.2	100.0	33.9
Maretts C 4–8	12.5	593	942	59	16.4	2.5	90.0	68.9
Cook 142–10	12.5	465	762	64	6.2	3.3	96.7	70.8
Dixie Triumph 366	12.5	392	832	112	20.0	3.3	100.0	89.7
Miller 610	12.5	295	862	192	63.9	6.3	100.0	96.9
Hurley's Rowden	12.5	157	594	279	84.3	38.0	100.0	98.9

[a] Controls (Con.) received no treatment.

There was an increased yield from treatment and almost complete control of wilt and root-knot. Growth differences began to appear early in the season, particularly with Deltapine. After 8 weeks, plants in treated rows were 3 inches taller than those on non-treated plots; and differences became greater as the season progressed. The yield differences in this and later tests were accounted for largely by number of bolls, although the bolls and seed were larger and the lint indices higher from treated plots.

FUMIGATION OF HEAVILY INFESTED SOIL

The study was repeated, with minor variations, at Tallassee, Alabama, in 1947. The soil used at Tallassee is a Cahaba fine sandy loam, deep phase. Cotton has been grown on this particular area for several years

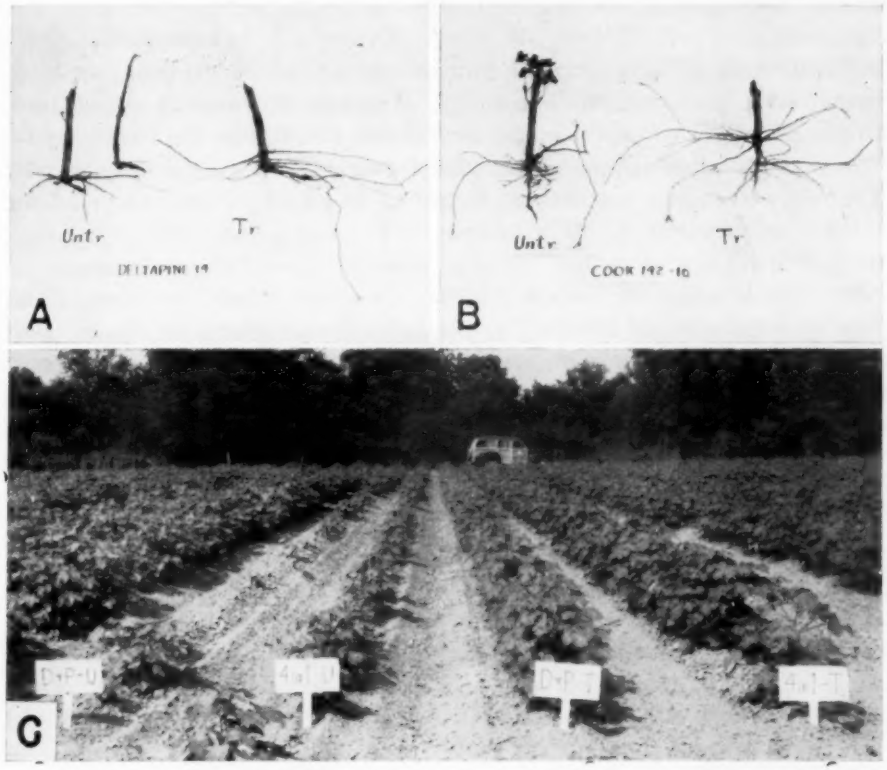

FIG. 1. A. Roots of Deltapine 14 cotton from untreated and treated plots; rate 37.5 gallons per acre. B. Roots of Cook 142–10 from untreated and treated plots; rate 12.5 gallons per acre. C. Early killing and stunting in untreated plots compared with vigorous growth in treated plots. Tallassee Test I, photographed July 10.

and the infestation of wilt, root-knot, and meadow nematode was heavy. Only the more resistant varieties can survive, and even these become stunted and wilted, and production is reduced. Otherwise, the soil is considered one of the best in Alabama for cotton production. The same varieties, Deltapine 14 and Coker 4 in 1–7, were planted. The test con-

sisted of 6 replications, with rows 70 feet long. The two treated rows were alternated with two non-treated rows to make up a replication. The fumigant was injected April 26 at a depth of 6 to 7 inches at staggered points 15 inches apart at the rate of 37.5 gal. per acre. The center row of injection points was in the row to be planted, with a row of injections 12 inches distant on each side. A guard row was not used between treated and non-treated rows and there was no evidence that the effect of the fumigant extended beyond the rows treated. The seed were planted under favorable conditions May 6, and an excellent stand was obtained for both varieties. Soil temperatures were not available for the period between soil treatment and planting, but the mean maximum and minimum air temperatures were 79° and 62° F., respectively. From this it may be assumed that the soil temperatures were favorable for the action of the fumigant.

Differences in rate of plant growth and amount of wilting were apparent early in the season (Fig. 1, C). Yields of lint were increased from 367 to 1067 lb. for Coker 4 in 1–7 and from 64 to 904 lb. for Deltapine 14. These yields represent increases of 191 and 1312 per cent, respectively. The wilt percentage was reduced from 52.7 to 1.8 and from 96.8 to 3.2 for the respective varieties. Wilt severity in this test is indicated by the high incidence in Coker 4 in 1–7, which is considered a relatively resistant variety in most soils. The root-knot indices were recorded November 26 and represent the seasonal build-up of the infestation. Plants in treated plots had normal appearing roots, indicating good control of both species of nematodes (Fig. 1, A). Such galls as were present were small and probably resulted from relatively late invasion of roots.

REDUCTION IN AMOUNT OF FUMIGANT

To determine the effect of a lower rate of application of the fumigant, an additional test was conducted at Tallassee, Alabama, in 1947. The rate used in this test was 12.5 gal. per acre. The material was injected to a depth of 6 to 7 inches at intervals of 15 inches in the row only. The varieties were randomized in 6 replications. A plot consisted of one row, 40 feet long. The number of varieties was increased to 6. Three varieties, Marett's C 4–8, Cook 142–10, and Coker 4 in 1–7 are considered wilt-resistant, and they have the best root-knot tolerance available in upland cotton varieties. Dixie Triumph 366 is resistant to wilt but rather susceptible to root-knot. Miller 610 is moderately wilt-tolerant and susceptible to root-knot. Hurley's Rowden is highly susceptible to both wilt and root-knot.

The yield data (Table 1) show much the same response to treatment as was obtained with the heavier application used at Tallassee. For the variety Coker 4 in 1–7, which was planted in both tests, the mean difference between plots in the two tests, 104 lb., may be partially attributed to

response to the heavier treatment. Plants were larger and grew more vigorously on the plots treated at the rate of 37.5 gallons.

The yields of other varieties having some wilt resistance were comparable to those of Coker 4 in 1–7 in treated plots. The small differences in yields are considered varietal rather than responses to treatment. Only the highly susceptible Rowden was not adequately protected to give the maximum expected yield in comparison with other varieties. The 12.5-gal. rate used in combination with resistant varieties appears about the optimum amount from the standpoints of economy of material and yield response.

The wilt was reduced to a negligible amount by treatment in all varieties except Rowden. Wilt percentages from 3 to 6 are not considered as factors affecting yield with good original stands, such as occurred at Tallassee. The roots of wilt-resistant plants in treated plots were protected sufficiently to permit good growth and production (Fig. 1, B). Root-knot indices in several varieties indicate a high infestation on the treated plots. Observation of the roots shows good protection as far as the fumigant extended. Largest galls were formed on roots extending beyond the limit of the effective range of the gas.

SUMMARY

Markedly increased yields and almost perfect control of cotton wilt and nematodes were obtained by the use of 31 and 37 gallons per acre of Dowfume W-10 as a soil fumigant with both resistant and susceptible varieties of cotton.

More economical control and comparable yields were obtained with applications of the fumigant at the rate of 12.5 gallons per acre applied in the row only. A variety highly susceptible to wilt and nematodes was not adequately protected at the 12.5-gallon rate of application.

ALABAMA AGRICULTURE EXPERIMENT STATION,
AUBURN, ALABAMA.

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A SIMPLIFIED METHOD OF PURIFYING TOMATO BUSHY-STUNT VIRUS FOR ELECTRON MICROSCOPY[1]

RUSSELL L. STEERE[2] AND ROBLEY C. WILLIAMS[3]

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INTRODUCTION

The application of electron microscopy to virus studies has allowed a new approach to some of the problems of virus identification. Uniformly sized particles, not found in controls, have been isolated by numerous workers from plants known to be infected with tobacco mosaic, tomato bushy-stunt, southern bean mosaic, and a few other virus diseases. These have been highly purified and, because of their intimate association with infectivity, are now considered to be virus particles. They will be so designated throughout this paper. Purified suspensions of particles of the above mentioned viruses have been photographed and each has been found to have a distinct shape and size.

Stanley (4) has mentioned the possibility of using the electron microscope for diagnostic purposes, but this procedure has not yet been generally adopted, because of the difficult purification procedures required.

Sigurgeirsson and Stanley (3) found it unnecessary to preserve the initial concentration of tobacco mosaic virus particles in the plant sap in order to locate them with certainty in the electron micrographs. Because of the rodlike shape and the large natural concentration of these particles, one part of expressed sap from infected plants to 50 parts of water resulted in identifiable specimen fields, and no special procedure appeared necessary for general observation and for non-specific identification of individual particles. Virus particles such as those of tomato bushy-stunt, on the contrary, do not appear as easily distinguishable rods, but rather as tiny spheres which are similar in size to many of the normal plant components. Also, the concentration of these particles in the infected sap is generally much less than that observed for tobacco mosaic, and a 1 : 50 dilution of the original plant juice scatters them so thinly that they cannot be photographed with certainty.

We are using the tomato bushy-stunt virus, grown in *Datura meteloides*[4] for an electron microscopic study of the mechanism of virus multiplication, and it is obviously desirable to subject the infected plant sap to as mild and brief a purification treatment as possible. Previously adopted purification methods have involved precipitation of the virus with ammonium sulphate, or concentration by high speed centrifugation, but we feel that

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[2] Department of Botany, University of Michigan.

[3] Department of Physics, University of Michigan.

[4] We are indebted to Dr. W. M. Stanley for the suggestion that *Datura* is a very satisfactory host for this virus.

these prolonged procedures are liable to remove whatever characteristic signs of virus reproduction might exist in the untreated sap. We have experimented with simplified techniques of partial purification until we are now able to purify the virus sufficiently for electron microscopic observations by means of a fairly gentle procedure requiring only about 25 minutes following the initial extraction of the sap.

PROPAGATION AND PURIFICATION

Seeds of *Datura meteloides* are germinated in sand and are transferred to sandy loam (20 plants in each 8-inch pot). Some of these pots are set aside for controls. The young plants in the others are inoculated when the first true leaves are about one-half inch across by gently rubbing the leaves with cheesecloth saturated with a mixture of one part crushed infected plants to 50 parts of distilled water and one part of fine carborundum. Carborundum is used to puncture the cell walls without injuring or having any toxic effect upon the cells. Plants are washed off with a stream of tap water immediately after inoculation. More than 95 per cent infection is regularly obtained in this manner.

Inoculated and control plants are placed in a specially constructed cabinet with controlled fluorescent lights and constant temperature of about 22° C. Three days after inoculation, numerous virus particles have always been found in the tips of plants thus infected.

The infected leaves and stem are cut and placed in 15-cc. glass centrifuge tubes where they are crushed and the juice pressed out by use of glass rods, the ends of which have been rounded by flaming. The expressed sap is transferred to clean tubes and diluted with an equal volume of distilled water. Heat coagulation (presumably of protoplasmic proteins and chlorophyll) is carried out as rapidly and at as low a temperature as possible. This is done by dipping the tubes containing diluted sap into boiling water and rapidly retracting and rubbing them with the hand to dissipate excess heat. This process is repeated until the first signs of coagulation appear at approximately 49° C. Coagulation is obtained in less than one minute. The tubes are held in the water bath no more than a total of 10 seconds, in contrast to approximately five minutes required when they are continuously immersed in a bath maintained at 50° C.

The coagulum is then sedimented, and loosely packed Celite (a diatomaceous earth) of approximately one-half the volume of remaining liquid is added. This is shaken, and again centrifuged at 3,000 r.p.m. for 3 minutes, after which the liquid is transferred to another clean tube and spinning is repeated for five minutes. Another transfer and spinning for 3 minutes is advantageous in eliminating the diatom particles. Drops of the final clear or slightly yellow-brown liquid are then placed on collodion or Formvar (polyvinal formal) films mounted on the usual electron microscope specimen screens. Each drop is allowed to remain in position over a screen for one-half minute and then as much liquid as possible is with-

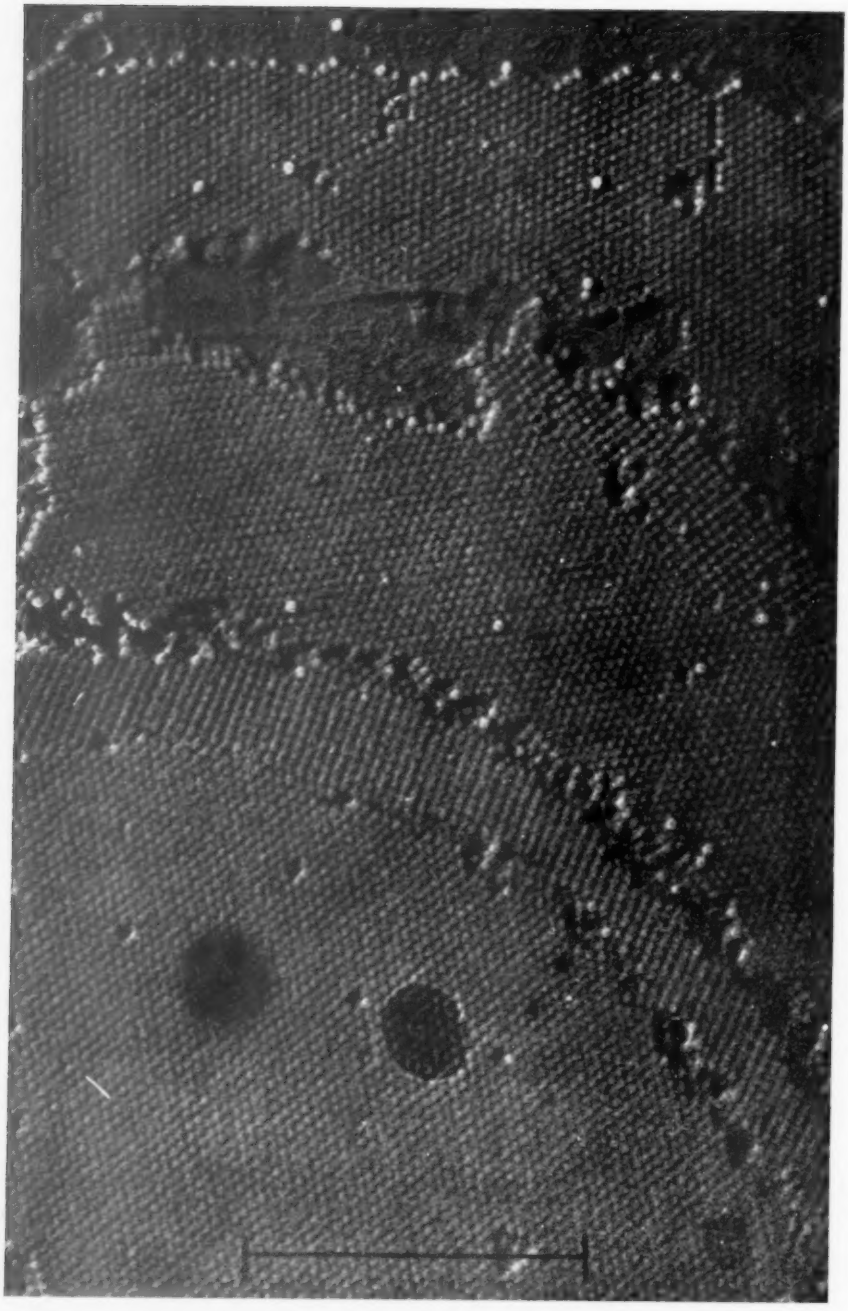

FIG. 1. Electron micrograph of the tomato bushy-stunt virus grown on *Datura meteloides*. Virus suspension allowed to dry on Formvar film after purification, and then shadow-cast with uranium. Ruled line shows one micron. Magnification is thus 45,000×.

drawn by a pipette. The remaining liquid is allowed to dry on the film. The screens containing this preparation have been shadow-cast with uranium at a 3-to-1 angle, using the technique of Williams and Wyckoff (5), and have been observed and photographed with an RCA type B Electron Microscope.

As a check, uninfected plants have been submitted to the same procedure as the infected. Whenever particles apparently similar in size to those found in infected material were seen with the electron microscope, they were photographed. In every instance the photographs indicated clearly that there were no spherical particles in the purified control material comparable to those which were always found in preparations from the infected plants.

Datura plants inoculated with the purified liquid, samples of which yield good photographic indication of virus particles, become infected in the usual manner. It would require comparable series of inoculations with successive parallel dilutions of processed and non-processed sap to determine conclusively whether or not the purification technique inactivates part of the virus, but rough experiments so far made do not indicate any loss of infectivity. Bawden (1) has compared the infectivity of unheated and heated virus preparations and has found nearly 50 per cent loss of infectivity with treatment at pH 6 and 50° C. for ten minutes. It is presumed that the short period of heating in the present procedure would have less effect than that observed by Bawden though the temperature of that part of the liquid adjacent to the tube wall may exceed 50°.

We have found it impossible to carry out the purification procedure without the addition of distilled water to the original sap. It is probable that if the normal plant proteins are coagulated from too concentrated a solution (or suspension) they carry down the virus particles with them. This might come about merely by mechanical entanglement with associated particles in a suspension so concentrated that interparticulate freedom of movement is not possible.

Purification by the method described is complete enough to permit the formation of flat layers of aggregates of uniform-sized spherical virus particles when Formvar films are used. A geometrical arrangement is observed in these aggregates (Fig. 1) which might be expected to result from particles of such uniform size that they adjust to that arrangement which utilizes space most economically, i.e. in a square or a hexagonal arrangement as viewed vertically. In some photographs, areas showing as many as three distinct layers have been observed (Fig. 2). When drops of virus suspension purified in the same manner are dried on collodion films, small aggregates among numerous rather evenly spaced particles result (Fig. 3).

Particles small enough to suffer considerable Brownian movement might by such movement adjust themselves to a regular geometrical or crystalline arrangement as they dry out of suspension. Since it may be assumed that

FIG. 2. Same preparation as shown in figure 1. Stratification of aggregates into three distinct layers is plainly visible. Magnification 45,000 ×.

FIG. 3. Preparation similar to that shown in figure 1, but allowed to dry on collodion film. Magnification 50,000 ×.

very slight forces of adsorption or of differential wetting might mask the Brownian motion forces and be sufficient to prevent the orderly arrangement of uniform particles of colloidal dimensions, we need not necessarily assume that the same results should be obtained with surfaces as different in their adsorbing behavior as collodion and Formvar appear to be. The results as indicated above seem to show that such a condition actually exists.

Price, Williams, and Wyckoff (2) have previously recorded crystalline structure of tomato bushy-stunt and southern bean mosaic virus particles following purification by differential ultracentrifugation.

SUMMARY

A new procedure requiring only small amounts of infected plant material has been developed for rapid purification by differential adsorption of ultramicroscopic particles from the sap of plants of *Datura meteloides* infected with tomato bushy-stunt virus preparatory to examination by the electron microscope.

The purification procedure involves: (a) coagulation of normal protoplasmic constituents by heating briefly to approximately 50° C., (b) adsorption of other normal components onto diatomaceous earth, (c) centrifugation at 3,000 r.p.m. at specific intervals to eliminate coagulum and diatom material, and (d) application of suspension to Formvar or collodion films and subsequent removal of excess liquid.

Suspensions thus obtained contain vast numbers of particles of uniform size which are completely lacking in control preparations from uninfected plants and are therefore interpreted as particles of bushy-stunt virus.

Such preparations are actively infective to *Datura*.

When suspensions of virus particles purified by this technique are dried on films of Formvar, electron micrographs reveal regular aggregates of crystalline appearance in which the lines of particles intersect at angles of 90° and 120°.

When the same preparation is dried on collodion, electron micrographs show only small aggregates among numerous rather evenly spaced particles.

UNIVERSITY OF MICHIGAN
ANN ARBOR, MICHIGAN

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CHEMICAL TREATMENT OF SOYBEAN SEED IN RELATION TO NODULATION BY NODULE BACTERIA[1]

M. F. KERNKAMP

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Along with the phenomenal rise of the soybean industry in Minnesota in recent years, problems concerning cultural practices are beginning to appear. One of the most pertinent questions at the present time is whether chemical seed treatment will kill nodule bacteria (*Rhizobium leguminosarum*) and thus nullify the beneficial effects of using a commercial nitrogen inoculant.

Preliminary results reported by Allington, Kent, Tervet, and Koehler (2) indicated that nodulation on soybean roots was slightly reduced by seed treatment in Alabama, but was not impaired in other areas.

Kadow, Allison, and Anderson (3) reported a reduction of nodulation when bacterial inoculant was applied to pea seed after treatment with Semesan, cuprous oxide, and zinc oxide, and sown in partially sterilized soil. They found no reduction in nodulation when similarly treated seed was planted in field soil where the nodule forming bacteria were already present. Likewise, Sharvelle, Young, and Shema (5) found no harmful effect of Spergon on nodule bacteria on peas in field tests.

Miller (4) found that Spergon had no effect on root nodulation in peanuts grown in the greenhouse either when seed was inoculated with commercial preparations of nodule bacteria or left uninoculated. Albrecht (1) compared yields of peanuts grown from nontreated and Spergon treated seed in plots that had been inoculated with nitrogen bacteria. His results indicated that Spergon was not injurious to nodulation.

The experiments reported here were designed to test in more detail the effects of seed treatments on nodulation in soybeans when chemical seed disinfectants and nodule bacteria were both applied to seed at different times or at the same time.

PROCEDURE

The following methods of seed treatment and inoculation were tested in the greenhouse: (1) seed treated with Spergon and not inoculated; (2) seed treated with Spergon 2 days after inoculation; (3) seed treated with Spergon and inoculated at the same time; (4) seed treated with Spergon and inoculated 2 days later; (5) seed inoculated but not treated with Spergon; and (6) seed not treated or inoculated. Each treatment was replicated three times and each experiment was repeated three times. One replicate comprised 10 plants in a 6-inch plot, thus giving 30 plants per treatment per experiment. The greenhouse tests were made with soil auto-

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claved for 4 hours, and with non-autoclaved soil, both experiments being run simultaneously.

Spergon (tetrachloro-para benzoquinone) was applied at the rate of 2 oz. per bu., and the inoculant, commercial Nitragin, was applied according to the recommended wet method. In all tests attempts were made to duplicate farm procedure insofar as possible.

The variety Habaro (Minn. No. 109) was used throughout. The number of nodules on the roots of plants were counted after they had grown for approximately 6 weeks. By that time the plants were approximately 18 inches high, and the soil was thoroughly penetrated by roots. The data were analyzed statistically by analysis of variance.

Preliminary results of a field experiment also are presented. In this test the plan was identical to that given above, except that New Improved Ceresan (ethyl mercury phosphate) and Semesan Jr. (ethyl mercury phosphate) were also tested on the varieties Ottawa Mandarin (Minn. Acc. No. 219) and Wisconsin Manchu No. 3 (Minn. Acc. No. 210).

RESULTS

Table 1 summarizes three greenhouse experiments in which soybean

TABLE 1.—*Summary of greenhouse experiments on the number of nodules on Habaro soybeans grown from seed inoculated with Nitragin bacteria, treated with Spergon, and planted in non-autoclaved soil*

Experiment and Date	Treatment and number of nodules[a]					
	Spergon				No Spergon	
	No Nitragin	Nitragin 2 days before Spergon	Nitragin with Spergon	Nitragin 2 days after Spergon	Nitragin	No Nitragin
1. April, 1947	55.7	46.6	62.3	46.3	28.6	52.0
2. June, 1947	234.0	283.0	237.3	322.7	274.6	334.0
3. March, 1948	15.3	56.6	39.0	66.0	72.0	16.3
Mean of all experiments[b]	101.7	128.7	112.7	145.0	134.4	125.1

[a] Each figure represents the average number of nodules on three replicates of 10 plants each.

[b] No significant difference between means when analyzed by analysis of variance.

seed was treated with Spergon, inoculated with Nitragin, and planted in non-autoclaved soil in the greenhouse. Nodules developed equally well regardless of whether or not inoculum was applied, and regardless of the time of application of inoculant and Spergon. The means of all three experiments varied only from 101.7 to 145.0 nodules per 10 plants for all treatments. Although there seemed to be a tendency for more nodulation when the inoculum was applied after treatment with Spergon, the differences between means were not statistically significant. It is assumed that nodule forming bacteria were already present in the soil prior to seed in-

oculation. These results are in agreement with those of Kadow et al (3), who reported that if pea seed was planted in soil already containing nodule bacteria, nodules developed regardless of inoculation or treatment with a chemical disinfectant.

There was considerable difference between experiments in degree of nodulation. The low number of nodules in the first experiment (Table 1) can be explained by the fact that the plants were given less water than is optimum for nodule formation. The discrepancies between the other expriments could be explained by the fact that different sources of soil and inoculum were used in the different experiments. The important thing is that the trend was the same in each experiment, and there was no significant difference between the over-all means.

Parallel experiments were made, along with those described, using par-

TABLE 2.—*Summary of greenhouse experiments on the number of nodules on Habaro soybeans grown from seed inoculated with Nitragin bacteria and treated with Spergon and planted in soil autoclaved for 4 hours*

Experiment and Date	Treatment and number of nodules[a]					
	Spergon				No Spergon	
	No Nitragin	Nitragin 2 days before Spergon	Nitragin with Spergon	Nitragin 2 days after Spergon	Nitragin	No Nitragin
1. April, 1947	1.0	6.3	5.0	36.3	4.3	1.0
2. June, 1947	23.7	47.6	83.7	67.3	491.0	15.7
3. March, 1948	201.7	269.3	299.7	275.6	338.3	82.0
Mean of all experiments[b]	75.6	107.7	129.4	126.4	277.9	32.9

[a] Each figure represents the average number of nodules on three replicates of 10 plants each.

[b] See table 3 for significant differences when analyzed by analysis of variance.

tially sterilized soil, autoclaved 4 hours. The results are presented in table 2, and the statistical significance of the data is presented in table 3.

As in the experiments with non-autoclaved soil, there was no statistically significant difference in nodulation when Spergon without inoculum was compared to Spergon with inoculum applied at different times. But when no inoculum and no Spergon were applied, there was a significant reduction of nodulation compared to inoculation at the same time as treatment and inoculation after treatment. When seed was inoculated without Spergon, nodulation was greatly increased over all other combinations of inoculation and application of chemical.

These results again indicate that Spergon used in combination with Nitragin, even in partially sterilized soil, did not significantly influence nodulation in soybeans. Nodulation was significantly increased, however, when Nitragin was applied without Spergon as compared to Nitragin with Spergon in any combination.

Here again differences will be noted between the data from different experiments. The reasons given for such variation in the experiments with non-autoclaved soil will apply here since they were made at the same time.

An experiment was made in the summer of 1947 to test the same methods of inoculation and seed treatment on nodulation and yield in the field. In addition to testing Spergon with inoculant, Semesan Jr. and New Improved Ceresan were tested. Also, two additional varieties, Ottawa Mandarin and Wisconsin Manchu No. 3, were included in the test. The experiment was laid out in randomized blocks, replicated four times. Nodules were counted on an average of approximately 15 plants per row on August 25, 1947, and yields were recorded at harvest time. The plot was planted on a piece of ground that had never been known to be planted to soybeans previously.

The results are based on only one year's data and are necessarily preliminary. There was no statistically significant difference in nodulation or yield regardless of what combination of inoculum and either of the three seed disinfectants was used.

TABLE 3.—*Significance[a] of data on nodulation given in table 2*

Spergon, no Nitragin	vs. Nitragin 2 days before Spergon	not significant
do.	vs. Nitragin with Spergon	do.
do.	vs. Nitragin 2 days after Spergon	do.
No Spergon, no Nitragin	vs. Nitragin with Spergon	significant
do.	vs. Nitragin 2 days after Spergon	do.
Nitragin, no Spergon	vs. All Others	do.

[a] Significance was determined by analysis of variance and comparing means, using L.S.D. = 90.1 at the 5 per cent level.

These results agree with the results in non-autoclaved soil, and they agree with reports of Kadow et al (3) and Miller (4), who both stated that nodulation is not affected by seed treatment or inoculation when seed is planted in soil that has not been partially sterilized or in soil where the nodule bacteria are already present.

DISCUSSION AND CONCLUSIONS

The application of Spergon with commercial Nitragin did not influence nodulation when the seed was planted in soil that already contained the bacteria; and preliminary results from one field experiment indicate that seed treatment with Spergon, New Improved Ceresan, or Semesan Jr. had no effect on nodulation or yield. Even in soil autoclaved for 4 hours the various combinations of Spergon with or without commercial Nitragin did not affect nodulation. However, results with steamed soil indicated that Spergon reduced nodulation as compared with the non-treated check. The latter may be interpreted to mean that Spergon may reduce nodulation when inoculated seed is planted in soil that contains a small number of nodule bacteria.

Only soil from the Experiment Station farm at St. Paul was used in

the tests, and the results obtained may not apply to other soil throughout the State or the soybean area of the Corn Belt. But with the widespread production of soybeans, it seems likely that nodule bacteria are also widespread, and therefore Spergon would not reduce nodulation in most soybean fields. This is supported by the fact that there were nodules on plants in one field that had never been known to have grown soybeans previously, and is further supported by results of other experimenters (1, 2, 3, 4, 5), who found no apparent deleterious effect of seed disinfectants when legume crops were planted in soil already containing the bacteria.

The time of application of inoculant or fungicide does not appear to be of major importance, since the degree of nodulation did not differ significantly with different times of application. However, in view of the fact that slightly more nodulation resulted when the inoculant was applied after the Spergon, even though the differences were not statistically significant, it is recommended that that method be followed. The directions that appear on the containers of the Spergon and inoculant appear to be adequate.

UNIVERSITY FARM,
ST. PAUL, MINNESOTA.

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BACTERIA IN THE STORAGE ORGANS OF HEALTHY PLANTS

IAN W. TERVET[1] AND JOHN P. HOLLIS[2,3]

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INTRODUCTION

It is generally believed that healthy plant tissue is free from microorganisms. This viewpoint draws most support from negative results obtained in the last century (2, 5, 15). A complete review of earlier work is furnished by Berthold (1). Evidence to the contrary is of comparatively recent origin (3, 6, 7, 9, 10, 11, 12, 13, 14). The most pertinent reports have all arisen since 1936 and are mostly independent of one another. Since this work is also of independent origin, no formal review of literature will be given.

The stimulus for the investigation reported herein arose from a chance discovery by the senior author of bacterial action in cultures of potato plugs submerged in sterile water. Breakdown of the plugs occurred and with this the liberation of whole starch which was deposited in the bottom of the culture tubes. Since the plugs of tissue had been removed aseptically, it was considered probable that the bacteria might be present inside the tubers. Experiments were conducted during the summer of 1947 to test this possibility.

MATERIALS AND METHODS

Tubers and storage organs of potato, carrot, turnip, red beet, and sweet potato along with fruits of tomato and kohlrabi buds were tested for the internal presence of bacteria. The organs were washed in tap water, placed in either 1:1000 acid mercuric chloride or calcium hypochlorite of 2 per cent chlorine strength for 5–20 minutes, removed, and blotted so as to remove excess moisture.

Plugs of tissue were removed by two methods. The first utilized a cork borer that was dipped in alcohol and flamed. A flap of tissue was torn from the surface of the organ to be tested, the borer inserted and pushed through to the opposite side. The surface tissue at the end of the borer was then extruded by means of a sterile glass rod and excised with a sterile scalpel. The plug of tissue was then pushed out into a sterile culture tube water blank. The second method utilized a scoop commonly employed in transporting chemicals for weighing. The tuber was cut part way, then broken into two halves. The scoop previously flamed red hot, cooled in 95 per cent alcohol and reflamed, was then inserted into the broken surface and a wedge-shaped piece of tissue removed. Figure 1, A shows plugs

[1] Camp Detrick, Frederick, Maryland, and formerly Associate Plant Pathologist, Nebraska Agricultural Experiment Station.

[2] Graduate Assistant in Plant Pathology, Nebraska Agricultural Experiment Station.

[3] Published with the approval of the Director as Paper No. 451, Journal Series, Nebraska Agricultural Experiment Station.

taken by the two methods. Cork borers used in the test were always autoclaved before the next lot was sampled.

The method of sampling must be noted. Tubers of freshly harvested Red Warba potato comprising 12 lots taken from Central Nebraska in August, 1947, were selected for large size and 5 plugs taken from each tuber transectionally. Likewise, 5 plugs were taken from kohlrabi buds, red beet, turnip, and carrot, and 3 plugs from green tomatoes. No particular pattern of plug removal was used in sampling other lots.

All cultures were incubated at room temperature. Over the period

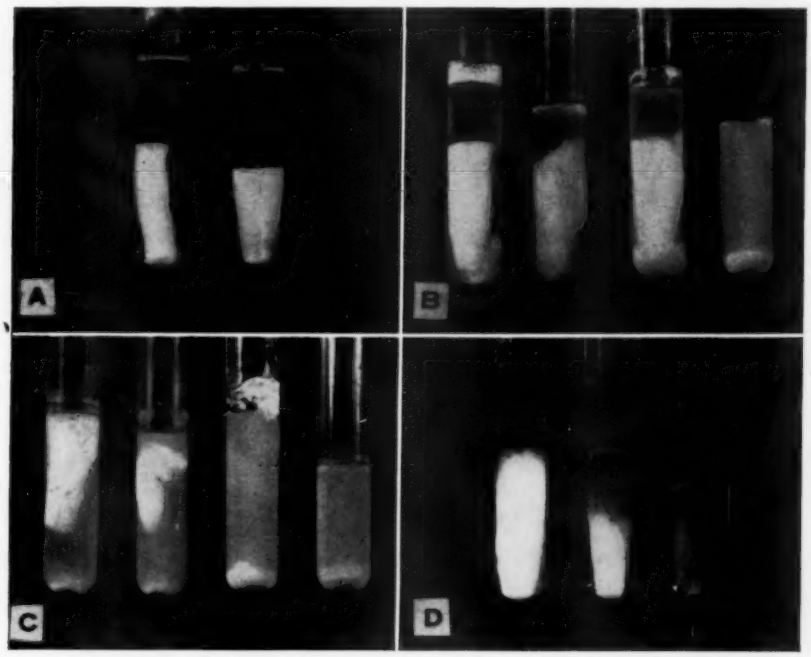

FIG. 1. A. The two types of aseptic potato plugs used in determining the occurrence of bacteria in plant tissue. B. Types of bacterial growth or tissue change found in aseptically produced potato plugs l. to r. turbidity, pellicle, green fluorescence, tissue breakdown. C. Stages in breakdown of potato plugs resulting in starch liberation. Left: plug in initial stages of breakdown. Center tubes: further stages in breakdown. Right: tissue digested leaving deposit of starch at bottom of tube. D. Three steps in the blackening of submerged potato plugs.

during which the work was performed, the average laboratory temperature was in excess of 30° C.

EXPERIMENTAL WORK

In the initial phases of this study it was noted that complete breakdown of "sterile" potato tissue submerged in sterile water with the liberation of whole starch grains from the cells was of rather infrequent occurrence. The most prevalent type of growth in submerged plug cultures was a uniform turbidity with gas formation. Turbidity with green fluorescence was less frequent but occurred with more regularity than complete break-

down. There was high correlation between certain lots of potatoes sampled in early tests and the presence of bacteria producing turbidity and gas. No such correlation existed between potato lots and the presence of organisms responsible for complete breakdown of potato tissue.

In order to learn more about the prevalence and distribution of bacteria in healthy potatoes, a number of experiments were conducted in sequence. Table 1 gives the results of these tests. The number of cultures

TABLE 1.—*Summary of bacterial isolation tests from healthy plant tissues using plugs of tissue removed aseptically and submerged in sterile water*

Plant and plant part	Lot No.	Method	Incubation time in days	Number of plugs	Sterile plugs	Cultural types of growth			
						Gas and turbidity	Gas and green fluorescence	Pellicle	Cellulose decomposition
Potato Tuber[a]	1–12	Borer	10	600	435	78	16	68	0
Do	13	Do	15	30	20	10	0	0	0
Do	13	Scoop	2	10	1	7	0	0	2
Do	13	Do	7	30	2	28	0	0	0
Do	13	Do	8	34	4	27	4	1	0
Do	13	Do	2	30	27	2	0	1	0
Do	13	Do	15	60	45	15	0	0	0
Do	14	Borer	4	24	1	0	0	0	23
Do	15	Do	3	70	38	26	0	6	0
Aerial tuber	16	Do	3	15	8	0	0	3	4
Do	16	Do	4	85	50	18	0	14	3
Do	16	Do	8	150	136	14	0	0	0
Kohlrabi Bud	1	Do	8	50	36	13	0	1	0
Red Beet Root	1	Do	8	50	30	20	0	0	0
Turnip Root	1	Do	8	50	9	41	0	0	0
Carrot Root	1	Do	8	49	34	15	0	0	0
Sweet potato Root	1	Scoop	7	30	30	0	0	0	0
Tomato Fruit	1	Borer	8	34	34	0	0	0	0

[a] Red Warba variety.

with turbidity and gas formation far exceeds the number with any other type of growth. Microscopic examination of turbid cultures revealed the presence of Gram negative rods although other types of bacteria frequently were present. Indeed, many cultures with initial turbidity were later found to possess a pellicle.

These cultures were always grouped with other pellicle-forming cultures and are enumerated this way in table 1. Figure 1, B shows the cultural types encountered. The few cultures in which there was complete

breakdown with starch liberation in this series of tests (Fig. 1, C) suggest that either the presence of these bacteria varies widely among lots of potatoes or that superficial contamination is responsible for the erratic results. It is significant that not one culture of this type was encountered in the large experiment involving 12 lots of potatoes. In this test the percentage of cultures in which growth occurred varied between 8 and 54 per cent for the various lots. Although 5 plugs were taken from each tuber there was no tendency for the bacteria to be distributed in tuber units. The converse was true in the case of carrots. No adequate explanation can be given of these results at present.

In the blackening of potato plugs in submerged culture, due to formation of melanin pigment (Fig. 1, D), there was complete agreement between tuber unit and the extent and intensity of color formation. The intensity and extent of blackening increased with the time beginning at the top and progressing toward the base. Some sets of 5 plugs remained completely white over the entire period of incubation.

Of other plant tissues from which isolations were made, turnip appears to be the most uniformly infested with bacteria. An examination of 10 cultures taken at random from tubes with growth in the turnip series revealed the presence of Gram negative rods in all 10 cultures. In addition to Gram negative rods, Gram positive rods of different size were found in one tube.

The industrial implication of cellulose breakdown with starch liberation made it desirable to conduct a preliminary investigation as to the nature of the organism bringing about this action. Microscopic examination of raw potato plug cultures in which breakdown was complete and starch was liberated revealed the presence of several morphologic types of bacteria. Uniformly present were large Gram positive rods, medium-size Gram positive rods, small Gram negative rods, and large clostridial forms.

It was possible to obtain pure cultures of the clostridial form by means of two successive heat treatments of aseptic potato plug cultures freshly inoculated with liquid from a broken-down culture. When these tubes were treated in water at 80° C. for one-half hour, the clostridial form survived this treatment and produced gas and turbidity after 24 hours. There was no breakdown by this organism alone. Morphologically these clostridial rods are variable. The ellipsoidal spores are borne terminally and are Gram positive. The vegetative part of the rod stains a light violet color with gentian violet. This part or "tail" is variable in length and diameter and may or may not be present with the spore.

The inability of pure culture transfers of this organism to raw potato plug cultures to bring about breakdown suggested that a complex of two or three organisms might be responsible for this action. Further tests were devised using potato-dextrose agar adjusted to a reaction of pH 7.0 as the test medium. Dilutions or loops of the original breakdown liquid

were used for all initial transfers to potato-dextrose agar. There was no growth in either potato-plug or Petri-dish cultures incubated in pure nitrogen indicating that at least one of the essential organisms was not a strict anaerobe. Inoculation of slants under sterile mineral oil, with liquid from a plug culture which had broken down, resulted in a whitish growth composed of the clostridial rod and small Gram negative rods which tended to be associated end to end in pairs. When a loopful of this growth was transferred to fresh, raw plug-cultures complete breakdown with starch liberation occurred in 48 hours at temperatures above 30° C. The optimum temperature for breakdown under the conditions imposed proved to be about 35° C.

Pure cultures of the Gram negative rod were obtained from slants by transferring the inoculum to dilution plates, which were then covered with a layer of sterile mineral oil, or by incubating the dilution plates under an oxygen tension of 10 mm./Hg. maintained by continuous faucet suction. Apparently the clostridial form required certain growth factors which were not carried over in sufficient quantities to dilution plates. It was noted also that successive transfer of the two organisms to fresh slants under oil resulted in loss of breakdown activity when these daughter slants were used for inoculating raw plug cultures. Obviously, the age of the slant culture may be important in loss of activity on potato-dextrose agar.

When the Gram negative rod was transferred to raw plug cultures, turbidity with gas formation resulted. The combination of both organisms was found necessary for breakdown. The complex was best retained in liquid from a culture which had previously broken down and at the time the work was terminated had retained viability in a sample of this liquid for about 5 months.

The identity of these organisms has not been pursued further. Muratova (8) obtained raw starch liberation from potatoes with the aid of an organism closely resembling *Bacillus felsineus* Carbone. Paziuk (10) described a single pleomorphic plectridial form which he showed capable of liberating raw starch from potatoes. It is not clear whether he was working with only one organism, because one type of rod could be seen in surface colonies while another type, a spore-forming plectridium, was found between the agar and glass at the base of the same culture. Buromski and Matiushenko (3) report breakdown of potatoes with starch liberation as due to a combination of cocci and rods.

The Gram negative rod reported in this work formed small, glistening white colonies 0.5–5 mm. in diameter. Most surface colonies were 2 mm. in diameter after 48 hours. The clostridial form reported herein is similar morphologically to the organism described by Muratova (8).

Further tests were conducted by inoculating the breakdown liquid from potato plug cultures into submerged tissue cultures of cooked potato, sweet potato, carrot, and alfalfa leaves and petioles. Cooked potato plugs broke

down completely with starch also disappearing. Breakdown in carrot and alfalfa occurred as with raw potato plugs. Sweet potato gave unique results in that cell separation occurred with no breakdown in individual cell walls.

DISCUSSION

From the standpoint of plant pathology, the most significant of these results is that pointing to the possible presence of a mixed bacterial flora in healthy storage organs. While bacterial types have not been identified, it is obvious that they may represent common soil forms. Sanford (12) cut thin sections from the stele in the stems of potatoes and beans from which he isolated a mixed bacterial flora. Taproots of sweet clover and alfalfa likewise yielded bacteria. He also isolated bacteria from the xylem of large potato tubers.

The method of isolation used in these tests was not sufficiently sensitive to detect the presence of bacteria in all healthy material tested; indeed, the majority of separate units yielded no bacteria. This indicates that the bacteria are present in small numbers.

Two principal types of entry portals are suggested by this and previous work. Sanford's results indicate that the bacteria are present in the vascular system of plants. This would suggest that bacteria may enter through natural wounds caused by emergence of secondary roots and that then they could be translocated to upper parts of the plant. Bacteria gaining entrance in this way should be present only in small numbers and their translocation into tubers then is a function perhaps of unusual external conditions. Bacteria may enter directly into storage organs through natural openings such as lenticels or through wounds and perhaps even through such protective barriers as periderm. If the latter hypothesis is true, we would expect to isolate bacteria more readily from surface layers than from stelar portions of storage organs and roots. Davidson (4) recently showed that bacteria of a soft-rot type gain entry through the stem end and eyes of the tuber and proceed along the pith strands into the tuber when these tubers are submerged in water for periods up to 5 days. Conceivably bacteria could enter tubers in this fashion prior to digging and effect no change in their healthy appearance.

As early as 1897, Zinsser (15) injected common species of bacteria into stems, roots, and leaves of various plants. He found that survival of certain forms occurred over a significant period but there was no multiplication of bacteria and very little movement from the point of inoculation. Berthold (1) found that bacteria and fungi would penetrate into vessels of a woody stem as a result of water intake from a cut surface. He found that survival of bacteria injected into healthy tissues occurred for as long as 10 months but no reproduction of adapted bacteria in the tissues was observed. Buromski and Matiushenko (3) as a result of working with starch-liberating bacteria of potatoes concluded that cocci and rods which

they found present in potato tubers could only develop intensively if the cells were ruptured by freezing or asphyxiated by submerging in water, both of which would result in death of the cells. Similarly, Romwalter and Király (11) concluded as a result of their isolations of yeast from gooseberries and grapes that a high carbon dioxide concentration promoted fermentation by yeast in healthy fruits, a process which did not occur if the fruits were maintained in air. They observed that, after fermentation of fruit in closed containers had run its course, the tissues were dead. This killing of cells was believed due to the presence of anaerobic bacteria. After restoration to air of berries in closed containers, the aerobic bacteria became active. It was considered that these were present inside the fruits and that conditions were made favorable for their growth by the previous decomposing activities of anaerobes.

These results show that furtherance of knowledge as to the occurrence and role of bacteria in healthy plant tissue may depend upon unusual methods of isolation. Obviously, bacteria can be isolated by bringing the organisms into contact with favorable nutrient substrates. The results above indicate the necessity, in some cases at least, of altering the natural substrate so that bacteria present in small numbers may develop in situ.

SUMMARY

1. Evidence is presented, from isolation studies involving more than 1000 submerged plug cultures, which points to the possible presence of bacteria in healthy storage organs of potato, red beet, turnip, carrot, and kohlrabi.

2. The results suggest that a mixed bacterial flora may be present.

3. Two morphologic types of bacteria comprising a clostridial form and a Gram negative rod were found to be responsible for breakdown of potato, carrot, and alfalfa tissue with liberation of cell contents, principally raw starch.

4. The two organisms were separated by means of heat treatments and dilution and grown in pure culture.

5. Blackening of potato plugs due to formation of melanin pigments occurred in characteristic fashion in individual tuber units.

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF NEBRASKA
LINCOLN, NEBRASKA

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MICROASCUS TRIGONOSPORUS FROM CEREAL AND LEGUME SEEDS

M. D. WHITEHEAD, M. J. THIRUMALACHAR, AND J. G. DICKSON

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During the course of studies on seed-borne fungi in barley, oats, wheat, and soybeans, isolations of a species of *Microascus* which resembled very closely *M. trigonosporus* Emmons and Dodge (2) were secured in great frequency. Since the seeds were surface sterilized before plating, it was of interest to study whether the *Microascus* species was of the nature of a pathogen. A review of literature indicated that two records of the occurrence of *Microascus* species had been reported in the United States: *M. intermedius* Emmons and Dodge on diseased strawberry roots in North Carolina (2) and *M. lunasporus* Jones isolated from the dermal infections on the human hand (3). The latter was similar to *M. trigonosporus* Emmons and Dodge (2) also secured from the dermal infections of the hand of a man in Puerto Rico. These reports of possible pathogenic nature of the fungus led the writers to study the isolates from barley, oats, wheat, and soybeans in some detail.

MATERIALS AND METHODS

The seed samples used in the present studies were collected in different localities in the United States. Some of these samples were a few months from harvest and others were as old as twenty-five years. The seed samples were divided down to approximately 100 kernels in each lot and were surface sterilized by suspending in coarse cotton cloth and dipping into a solution of 1 part 95 per cent ethyl alcohol and 2 parts Chlorox solution (5.25 per cent sodium hypochlorite). The treatment period in the hypochlorite solution was one minute for barley, soybeans, and wheat, and 30 seconds for oats. The surface sterilized seeds were plated on potato-dextrose agar at the rate of ten seeds per Petri dish and incubated at 20° C. for 4–6 days. Observations for the developmental stages of conidia and perithecia were made at various intervals.

ISOLATION AND MICROSCOPIC STUDY OF THE FUNGUS

The first series of isolates of *Microascus* were made from "Mammoth yellow" soybeans grown in Alabama and stored longer than six months. The grayish-white colonies of the *Microascus* developed around the surface-sterilized seeds. In a similar manner barley, oats, and wheat kernels yielded the characteristic colonies of the *Microascus* when they were surface sterilized and plated on potato-dextrose agar (Fig. 2, A, B). In one sample of barley that had been stored in bottles in the laboratory longer than 25 years, profuse development of the *Microascus* colonies was noticed

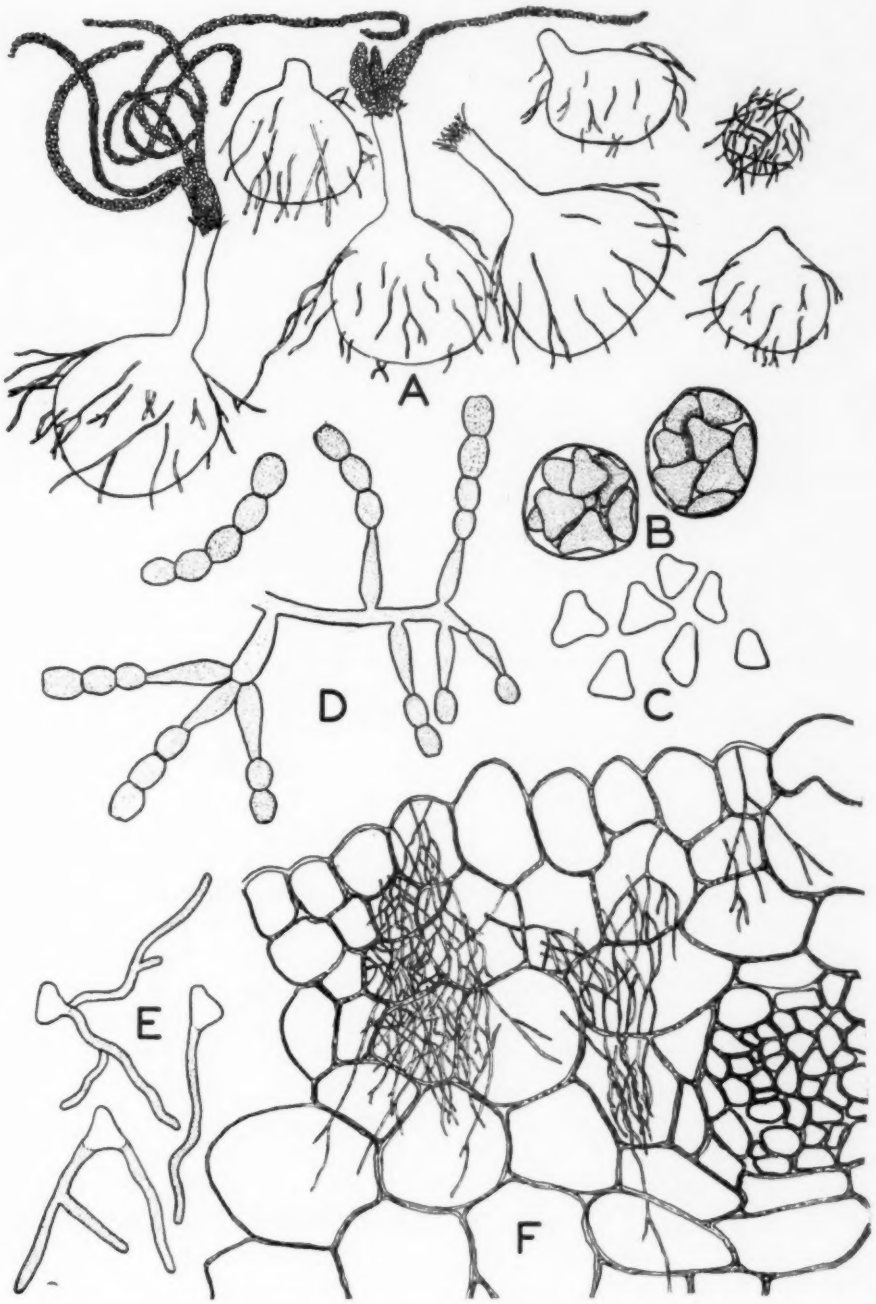

FIG. 1. Mycelium, conidia, and perithecia of *Microascus trigonosporus*. A. Perithecia of different maturity, some with ascospore tendrils (×300). B. Globular asci (×2000). C. Ascospores (×2000). D. Conidiophores and chains of conidia (×1500). E. Germinating ascospores (×1500). F. Mycelium within host tissue (×1320).

on all kernels (Fig. 2, A). The *Microascus* has been isolated from seeds secured from the following places in the United States:

Soybeans (Mammoth yellow variety)—Alabama.

Barley—Brookings, South Dakota; Fargo, North Dakota; East Lansing, Michigan; Mt. Morris and Urbana, Illinois; Crookston, Waseca, and St. Paul, Minnesota; Ames and Kanawah, Iowa; Madison, Wisconsin.

Oats (Vicland)—Madison, Wisconsin; Thermopolis, Wyoming.

Wheat—Madison, Wisconsin.

The fungus apparently is distributed widely in the United States.

The fungus on potato-dextrose agar first formed dull, white colonies composed of fine and closely interwoven hyphae that imparted a bacterioid consistency to colonies. As development proceeded, hyphal branches grew erect and developed conidiophores and catenate conidia characteristic of *Scopulariopsis* Bain., which has been shown by previous investigators (1, 2, 3) to be the conidial stage of *Microascus*.

The several isolates from barley, wheat, oats, and soybeans were compared morphologically. They were found similar and it was evident that they belonged to the same species. The life cycle of the fungus was studied in culture. The conidial stage developed first with conidia produced on aerial hyphae. Conidiophores were simple or branched and bore at the tip a single sterigma or verticil of sterigmata (Fig. 1, D). Conidia were formed in catenulations that either separated early or remained firmly united for a long time. Spores were ovate to lemon-shaped, smooth, and measured 2.5–3.3 × 4–5.5 μ.

Perithecia formed soon after development of conidia, appearing first as scattered, minute black specks. As perithecia developed in large numbers, the colonies turned black. The early stages of the development of the ascocarp have been given in detail by Emmons and Dodge (2) and Jones (3). Young perithecia were yellowish-brown with lax hyphae enveloping them; as they matured they turned carbonaceous black. The ostiole was well developed (Fig. 1, A) and the ostiolar beak varied from a mere protuberance to an elongated neck and resembled those figured by Curzi (1) for *Microascus cirrosus* Curzi. Arrangement of the asci within perithecia was characteristic of the Plectascales such as those occurring in the genera *Penicillium, Aspergillus,* and *Thielavia* as mentioned by Emmons and Dodge (2) and Curzi (1). The mature ascus was globose to spherical, with eight triangular ascospores (Fig. 1, B) that were released when the wall of the ascus deliquesced. Mature ascospores were triangular to slightly crescentric (Fig. 1, C; and Fig. 2, C), reddish brown, 3.0–3.5 × 4.5–5.2 μ. They were discharged in long cirri bound together by a gelatinous matrix as in some of the other species. In old drying cultures, some of these spore tendrils were several millimeters long and very conspicuous (Fig. 1, A). The shape of the ascospores has been over-emphasized in some of the species

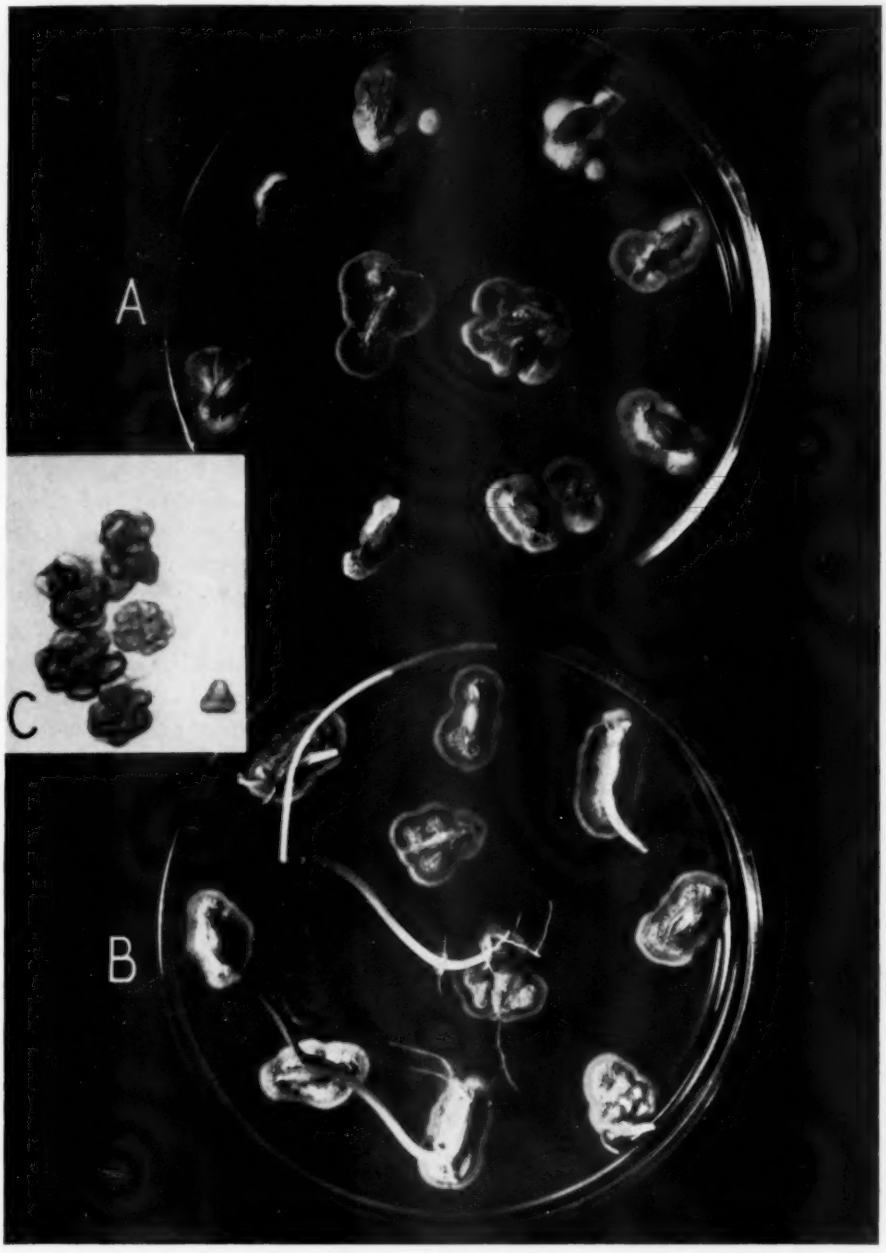

FIG. 2. *Microascus trigonosporus* from barley and oats. A. Platings of 25-year-old barley seed with colonies of *Microascus* from the seeds (Natural size). B. Platings of freshly harvested and naturally infected oats (Natural size). C. Photomicrograph of asci and ascospores (About 750×).

described. While the elliptic ascospores of *M. sordidus* are distinct from the triangular spores of *M. trigonosporus,* there is very little difference between the lunate spores of *M. lunasporus* and *M. cirrosus.* Since the *Microascus* species under study resembled *M. trigonosporus,* in most of the essential characters, it is proposed to refer the isolates described here to that species. *M. trigonosporus* has been known so far only from the type locality (Puerto Rico) where it was isolated from dermal infection of man.

THE FUNGUS IN RELATION TO HOST TISSUE

Repeated isolation of mycelium of the *Microascus* from surface sterilized seeds, indicated that the fungus was within the seed tissues. Studies were undertaken to determine the location of the fungus in the tissue and its early development. Seed externally infected with the fungus showed no evidences of disease symptoms, either in the dormant state or during germination. The staining methods used to locate dormant mycelium within tissues of dry seeds were unsuccessful. Kernels, surface sterilized and plated as described previously, were sectioned and stained with cotton-blue lactophenol after 3 days' incubation at 20° C. Hyphae were located in the tissues of floral bracts and pericarp. In the pericarp, hyphae were concentrated as interwoven masses three to four cell layers beneath the epidermis. Since the hyphae were growing rapidly, ramifying branches were observed easily in spite of their delicate nature. In cells of floral bracts, hyphal strands were more numerous and generally distributed.

The fungus invaded seedling tissues causing only slight macroscopic evidence of infection. On agar plates, mycelium developed on the surface of roots and especially coleoptiles of germinating seedlings. Light cinnamon-brown, amorphous substances within and between cells were evident in sectioned material. Sections of coleoptiles showed penetration of the epidermis and establishment of mycelium in the tissues (Fig. 1, F). The fine hyphae ramified rapidly, both inter- and intracellularly through the parenchyma. The fungus entered and ramified freely in cortical tissue around secondary roots. Hyphae appeared in small aggregates in the cortex adjacent to the stele. Further development in seedlings was not followed. Seedling inoculations gave no evidence of definite necrosis or reduced vigor among seedlings of cereals or soybeans.

Seed infection was studied by inoculating florets of barley and oats. The seed-borne nature of the fungus was established by plating and from sections of the cereal kernels. Barley and oat flowers at anthesis were inoculated with a water suspension of ascospores. Spore suspension was placed inside the floral bracts, without injury to the developing ovary, by means of a hypodermic needle; the inflorescences were enclosed in paper bags until the kernels were mature. Controls using sterile water were included in the series. Kernels from the inoculated flowers were well developed and showed no external evidence of infection.

The ripe grain was surface sterilized and plated. In one half of each lot the lemma and palea were removed completely; these structures and the naked caryopses were surface sterilized and plated on potato-dextrose agar. In the other half the hulled kernels were surface sterilized and plated. After seven days at 20° C. colonies of *Microascus* had developed from all kernels and floral bracts plated.

The fungus apparently persists within the enveloping tissue in dormant seeds of several crop plants. Blossom inoculation establishes the fungus in pericarp tissues and it is probable that natural infection occurs in a similar manner. Mycelium penetrated and ramified within the tissues of seedlings with only slight necrosis of the invaded tissues and no apparent damage to host development. This type of infection may be characteristic of a group of fungi commonly plated from seed.

WISCONSIN AGRICULTURAL EXPERIMENT STATION,
MADISON, WISCONSIN.

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THE EFFECT OF SOAKING COTTON SEED ON THE INCIDENCE OF ANGULAR LEAF SPOT IN NEW MEXICO AND ARIZONA

A. R. Leding and L. A. Brinkerhoff[1,2]

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Seed disinfection by water-soaking was suggested by Boughey[3] as a possible substitute for chemical disinfectants in controlling angular leaf spot of cotton in Egypt during the recent war. Primary infection was greatly reduced in the Egyptian studies when contaminated seeds were soaked in irrigation water for 48 hours. With Boughey's experiment in mind, water-soaking was included in angular leaf spot control tests at State College, New Mexico, and at Sacaton, Arizona, during the summer of 1942. As the first year's results were not in agreement with the Egyptian experiments the tests were repeated at both stations.

MATERIALS AND METHODS

At Sacaton, Arizona, naturally infested seed of the American-Egyptian variety S × P, *Gossypium barbadense* L., was used for Test 1, and the related variety Pima for Test II. Seed of the latter was sprayed with a culture of *Xanthomonas malvacearum* (E.F.Sm.) Dowson and then thoroughly air dried. For Tests III and IV at Sacaton and Test III at State College, New Mexico, seed of S × P was similarly inoculated. Seed of the upland variety Acala 1517, *G. hirsutum* L., obtained from naturally infected plants served for the remainder of the tests at State College.

The various lots of seed were soaked at room temperature and then air dried. The 48-hour period recommended by Boughey caused sprouting at the temperatures which prevailed at Sacaton, Arizona, during April of 1942 and 1943; consequently, the soaking period was reduced to 24 hours in April and to 12 hours in May. At State College where lower spring temperatures prevail, the 48-hour period was employed. Seed for Test III at both stations came from the same lot and was soaked at Sacaton for 24 hours. A 5-minute and a 1-hour soaking period were included in Test IV at State College in 1944.

The tests were conducted in the field and the seedlings grown under ordinary farm practices, each station using methods adapted to its area. Pre-planting irrigations supplied moisture for germination and growth of the seedlings for the duration of the tests except for Test IV at State College, which was irrigated before the final count was taken.

The percentages of plants infected with angular leaf spot (Tables 1

[1] Agronomist and Associate Pathologist, respectively, Division of Cotton and other Fiber Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, United States Department of Agriculture.

[2] The plantings at the United States Field Station, Sacaton, Arizona, were supervised by the late C. J. King.

[3] Boughey, A. S. Cotton seed disinfection in wartime. Nature **149**: 50–51. 1942.

and 2) are believed to represent primary infection in most instances. Where there remains a possibility that infection may have resulted from inoculum borne by rains or irrigation water this is indicated in footnotes accompanying each table. Three replications were used except in Tests I and II at State College which contained 2 and 5, respectively.

TABLE 1.—*The effect of soaking cotton seed on the incidence of angular leaf spot in cotton seedlings at the United States Cotton Field Station, State College, New Mexico*[a]

Test number, period, and treatment[b]	Number of seedlings	Percentage of seedlings infected with angular leaf spot
I.[c] *April 18 to May 23, 1942*		
Soaked 48 hours	2221	30.3
Not soaked	1773	4.1
II.[c] *May 1 to May 30, 1942*		
Soaked 48 hours	346	58.1
Not soaked	350	9.7
III.[d] *April 21 to May 27,1943*		
Soaked 48 hours	1648	28.1
Not soaked	1264	1.3
IV.[e] *May 11 to June 10, 1943*		
Soaked 48 hours	1604	24.2
Not soaked	889	0.9
V.[d] *April 21 to May 26, 1943*		
Soaked 24 hours (clear water)	188	26.6
Soaked 24 hours (silty water)	199	15.6
Not soaked	177	0.6
VI.[f] *May 19 to June 19, 1944*		
Soaked 48 hours	217	18.0
Soaked 1 hour	205	10.0
Soaked 5 minutes	211	8.5
Not soaked	194	1.0

[a] The data represent a summation of 2 replicates for Test I, 5 replicates for Test II, and 3 replicates for each of the remaining tests.

[b] Naturally infested seed of the upland variety Acala 1517 was used for all except Test V which was planted with artificially inoculated seed of the American-Egyptian variety S × P.

[c] There was no rain between seedling emergence and the date the final counts were taken. All infection is considered primary.

[d] Rains occurred as follows: May 20, .02 inch; May 22, .06 inch. Probably little secondary infection occurred.

[e] Rains occurred as follows: May 20, .02 inch; May 22, .06 inch; May 29, .67 inch; May 30, .05 inch. Irrigation water was applied June 9. Secondary infection probably occurred.

[f] Rains occurred as follows: May 27, .39 inch; June 6, .40 inch. Secondary infection probably occurred.

RESULTS

Results of six trials at State College, New Mexico, are shown in table 1; table 2 presents the results obtained at Sacaton, Arizona. In all instances at State College soaking caused marked increases in the incidence of angular leaf spot, and this was also true for two of the four tests at Sacaton. The primary lesions tended to be more numerous in the seedlings from soaked seed. Angular leaf spot failed to develop in seedlings from nonsoaked seed in Test III at Sacaton in 1943, a very dry spring. The seed soaked in clear water, however, produced 16.5 per cent infected seedlings in the same test.

TABLE 2.—*The effect of soaking cotton seed on the incidence of angular leaf spot in cotton seedlings grown at the United States Field Station, Sacaton, Arizona*[a]

Test number, period, and treatment[b]	Number of seedlings	Percentage of seedlings infected with angular leaf spot
I.[c] *April 1 to May 3, 1942*		
Soaked 24 hours	39	69.2
Not soaked	45	26.7
II.[c] *April 1 to May 3, 1942*		
Soaked 24 hours	590	1.2
Not soaked	832	0.1
III.[d] *April 14 to May 11, 1943*		
Soaked 24 hours (clear water)	683	16.5
Soaked 24 hours (silty water)	760	6.2
Not soaked	766	0.0
IV.[d] *May 10 to June 6, 1943*		
Soaked 12 hours	1425	0.2
Not soaked	2008	0.0

[a] The data represent a summation of 3 replicates for each test, except Test I.

[b] Test I was planted with naturally infested seed while Tests II, III, and IV were planted with artificially infested seed. The American-Egyptian variety S × P was used for all but Test II which was planted with the related variety Pima.

[c] Rains occurred as follows: April 12, .10 inch; April 21, trace; April 22, .10 inch; April 23, .40 inch; April 24, .67 inch; April 29, trace. Some secondary infection probably occurred.

[d] No rain fell for the duration of the test. All infection is considered primary.

The use of silty flood water resulted in a lower percentage of the disease than did the clear water at both stations; nevertheless, there was still a significant increase over the nonsoaked seed. The two shorter soaking periods included in Test VI at State College caused increases in infection which were intermediate between the results for the check and the 48-hour soaking.

DISCUSSION AND CONCLUSIONS

Boughey[4] notes that according to a previous author (Massey, Rep. Gezira Agric. Res. Serv. 1933, 1934), the angular leaf spot organism is destroyed when cotton fields are flooded for four days, this action being attributed by that author to the action of a bacteriophage. Boughey himself, however, says, "From laboratory experiments it would appear that the organism disappears from the surface of the seed, not through the activity of a bacteriophage, but through exposure to anaerobic conditions. These conditions are the result of bacterial activity and oxygen absorption by the germinating seeds. The growth of *B. malvacearum* in culture is closely conditioned by the amount of oxygen present." Whatever the agent or mechanism effective in the Egyptian tests, it was obviously not operative under the conditions that prevailed in Arizona and New Mexico.

The fact that water-soaking cotton seed tends to increase the primary infection of angular leaf spot in Arizona and New Mexico may at times be of economic significance. Growers occasionally soak fuzzy cotton seed be-

[4] *Op. cit.*

fore planting to insure rapid germination. The tests reported in this paper indicate that angular leaf spot may be considerably increased by this procedure.

UNITED STATES COTTON FIELD STATION,
STATE COLLEGE, NEW MEXICO,
AND
UNITED STATES FIELD STATION,
SACATON, ARIZONA.

AN EVALUATION OF CERTAIN SUBSTITUTED PHENOL ESTERS FOR THE TREATMENT OF COTTON SEED[1]

C. H. Arndt

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One of the objectives in recent experimentation with fungicides has been the discovery of a chemical that would be less irritating to the operator and less toxic to animals than most of the fungicides now in general use. This applies especially to the treatment of cotton seed, for which there are frequently surpluses of unplanted treated seed that could be profitably used for oil extraction. Preliminary tests have indicated the possibility of the extraction of marketable oil from cotton seed treated with zinc 2,4,5-trichlorophenate; while laboratory tests (8) and field plantings (4) indicate that it is an effective fungicide for seed treatment. The irritating properties of commercially available dusts that contain this chemical may cause some inconvenience in their use; nevertheless, the hazards associated with their use are relatively small. This paper presents results of studies made to ascertain whether available esters of 2,4,5-trichlorophenol and related phenols might be effective fungicides and be less irritating to the operator when used for the treatment of cotton seed.

The seed of the cotton plant when infested by *Colletotrichum gossypii* South. supply an excellent criterion of the effectiveness of a fungicide, since (a) seed copiously infested by this pathogen are usually available, and the virulence of the fungus is maintained when the seed are stored at 1°–3° C. (2), (b) seedling infection by this pathogen is readily obtained under standardized conditions (1), and (c) the lint remaining on the seed after ginning makes it essential that an effective fungicide be volatile to some degree.

METHODS

Results obtained when seed infested by *Colletotrichum gossypii* were germinated on water agar (1) and unpublished results obtained when similar seed were grown in sand culture have shown that a soil temperature of 24° C. approximates the optimum for the maximal infection of seedlings by this pathogen. Accordingly, treated seed were germinated in sand cultures that were placed in cases in which air temperature was maintained at 24.5° C., while the sand temperature was generally about 0.5° C. lower, because of evaporation from the cultures.

The data for seedling emergence and infection for each chemical are based on a triplicate planting of 32 seeds. Each group of 32 seeds was grown in an aluminum container, 18 cm. in diameter and 10 cm. deep, into which was placed 2500 gm. of steamed river sand. The water holding capacity (field capacity) of the actual mass of sand, as used, was 31.3 per

[1] Technical contribution No. 154 of the South Carolina Agricultural Experiment Station.

cent. Water was added to obtain a moisture content of 80 per cent of this amount to assure that there would be adequate aeration for rapid germination. The water added, 625 cc., contained mineral nutrients in the following concentrations: KNO_3, 0.002 M; $NH_4H_2PO_4$, 0.001 M; $Ca(NO_3)_2$, 0.004 M; and $MgSO_4$, 0.001 M. After the water was added, the sand was thoroughly stirred with an icepick and its surface leveled. Preliminary experimentation had shown that a thorough stirring of the sand is essential for rapid and uniform seedling emergence, probably because of the increased aeration of the sand. A planting guide into which 32 regularly spaced 12-mm. holes had been drilled was placed on the surface of the sand; one seed was dropped into each hole; and the seeds were finally forced into the sand to a depth of 2.5 cm. with a wooden punch 10 mm. in diameter. The seeds were covered with sand by stirring its surface with an icepick.

A relative humidity of about 75–80 per cent was maintained in the cases and the water loss from each culture after 7-8 days amounted to 100 to 150 cc. At this time the sand was brought to the initial moisture content by sprinkling water on its surface. Preliminary tests had indicated that the addition of water at this time with the associated packing of the moist sand around the bases of the hypocotyls tended to increase the number and severity of the anthracnose lesions, which improved the evaluation of the chemicals under test.

The cultures were not illuminated until after seedling emergence was almost completed, usually the 5th day, after which the cases were irradiated by three, 40-watt fluorescent lamps 10 cm. above the glass tops of the cases, or about 70 cm. above the seedlings. Irradiation did not seem to influence the final results, but did induce a more normal type of seedling growth. Seedling counts were made after 4, 5, 6, 7, 10, and 12 days to observe any effect of treatment on seedling emergence and on the severity of infection. On the 12th day the seedlings were removed from the sand to obtain data on seedlings that had lesions on the hypocotyls. Since 12 days were sufficient for maximal seedling infection but not for maximal injury to seedlings, no additional information would have been obtained by a longer growth period. Since the earlier counts showed no definite effect for any chemical at non-toxic dosages on the rate of emergence, the data, unless otherwise specified, indicate the total number of seedlings that were infected on the 12th day. Control cultures of untreated seed were grown in each test. The number of emerged seedlings was generally about the same as that for the more effective fungicides, but the number of uninfected seedlings was small, ranging from 0 to 6 for the particular lot of fuzzy[2] Delfos 651 seed used. This seed was stored at 3° C. until treated with the chemicals.

For convenience in making comparisons among the chemicals studied, all dosages are reported in grams of the active chemical per kilogram of

[2] Seed from which the longer seed-coat hairs have been removed in ginning, but not the shorter hairs that constitute the linters.

seed, although the chemicals were diluted with the requisite amount of micronized pyrophyllite to permit the application of the dust at a rate not less than 3 gm./kg. of seed, a rate application well below the maximal that would be retained by seed. The chemicals,[3] when initially prepared, were mixed with an equal weight of micronized pyrophyllite, after which the mixture was ground in a ballmill until a suitable dust was obtained. All dusts adhered well to the seeds. Other dilutions were obtained by adding the requisite amount of prophyllite to the 50 per cent dust. After the requisite amount of dust was added to the seeds (usually 200 gm.), the seeds were agitated for 10 minutes in a mechanically rotated churn. The seeds were then stored in a paper container at 20°–26° C. for 24 hours before planting.

The comparatively sharp melting points of most of the chemicals used, table 1, would indicate that they were relatively pure. This does not apply to the 2,3,4,6-tetrachlorophenyl acetate, which yielded materials of widely different melting points on recrystallization. It is assumed that the effects reported are due to this chemical, but the possible toxicity of impurities is not precluded. Although the planting unit for a single test of a given dosage of each chemical was a triplicate planting of 32 seeds, most of the results were confirmed by comparable data from two or more tests, particularly at the 2 gm./kg. dosage.

Statistical analyses of the data showed that differences in emergence of less than 15 per cent were not significant at the 1 per cent level. This relatively high difference required for significance was largely due to the difficulty of detecting all manifestly defective seeds before planting. Since the criterion of fungicidal effectiveness in these tests was the protection afforded seedlings against infection by *Colletotrichum gossypii*, the original data were recalculated to show the percentage of infection. Analyses of these percentages showed that a difference of 5.5 per cent was significant at the 1 per cent level.

RESULTS

A much greater effectiveness in reducing seedling infection by *Colletotrichum gossypii* for the formate, acetate, and propionate esters of 2,4,5-trichlorophenol than for the laurate, benzoate, succinate, and carbonate esters is indicated by the data on seedling infection, table 1. The results for the benzoate may not be entirely comparable to those for the other esters, since the dust prepared from this chemical was somewhat less dusty than that prepared from the other chemicals.

The position and number of the chlorine substituents influenced the fungicidal activity to a lesser degree than did the acidic radical. Thus, the 2,4,6-tri- and 2,3,4,6-tetrachlorophenyl acetates were almost as effective as the 2,4,5-ester; while the 2,3,6-tri- and pentachlorophenol esters were slightly less effective. Similarly, the substitution of bromine for chlorine

[3] Chemicals were supplied by Dr. W. S. Gump, Givaudan-Delawanna, Inc., New York, N. Y.

in the 2,4,5- and 2,4,6-trichlorophenyl acetates did not greatly affect the fungicidal activity.

Seeds treated with Ceresan-M (7.7 per cent ethyl mercury p-toluene sulfonanilide) and Dow 9B (50 per cent zinc 2,4,5-trichlorophenate) at a rate of 3 gm./kg. were also included in several of the tests. The emergence for seed treated with either of the dusts averaged about 75 per cent, and 2 to 4 per cent of the seedlings were infected by *Colletotrichum gossypii*. This percentage of infection is without significance, since a comparable test with acid-delinted seed indicated that a small percentage of

TABLE 1.—*Results from the germination in steamed sand of 96 cotton seeds infested by Colletotrichum gossypii after treatment with various phenyl esters. Dosages given indicate grams of active chemical per kilogram of seed*

Chemical	Melting point °C.	Seedlings[b]		Dosages	
		Emerged	Infected	Tested	Toxic[c]
1. 2,4,5-trichlorophenyl formate	72– 73	69	3	2–4	4
2. Do Do acetate	67– 68	75	1	0.5–4	3
3. Do Do propionate	59– 63	73	8	2–4	2
4. Do Do laurate	60– 61	74	44	2–3	.....
5. Do Do benzoate	85– 88	72	21	0.75–3.0	3+
6. Bis(2,4,5-trichlorophenyl) carbonate	170–171	57	36	2–8	8+
7. Bis(2,4,5-trichlorophenyl) succinate	138–139	53	47	2–3	.....
8. 2,4,5-tribromophenyl acetate	98–100	71	2	0.32–3	3
9. 2,3,6-trichlorophenyl acetate	40– 42	72	11	2	.....
10. 2,3,6-trichlorophenyl benzoate	89– 91	69	51	2	.....
11. 2,4,6-trichlorophenyl acetate	49– 52	65	2	0.5–4	2
12. 2,4,6-trichlorophenyl benzoate	71– 74	73	46	2	.....
13. 2,4,6-tribromophenyl acetate	82– 85	73	12	2–4	4
14. 2,3,4,6-tetrachlorophenyl acetate	70–110[a]	76	3	0.25–2	0.72–1
15. Pentachlorophenyl acetate	148–151	59	9	2–4	2
16. o-nitrophenyl acetate	37– 39	73	43	1–2	2
17. p-nitrophenyl acetate	77– 80	71	16	0.5–2	2
18. 2,4-dinitrophenyl acetate	71– 73	72	20	2	2
19. 4-chloro-3,5-dimethylphenyl acetate	47– 48	73	16	2	.....

[a] Prepared from technical grade chemical, Dowicide No. 6. Recrystallization of this chemical did not result in a product with a definite melting point.

[b] Dosage 2 gm./kg. of seed; except 1 gm./kg. dosages for Nos. 8, 16, and 17; and 0.5 gm./kg. for No. 14, which chemicals were toxic at the higher dosages.

[c] Toxic to cotton seedling as indicated by stunting or discoloration of hypocotyl.

the seeds were internally infected by this fungus; and no fungicide thus far tested has prevented the growth of this fungus on seedlings that develop from internally infected seeds.

The dosages at which the chemicals were toxic to the seedlings varied from 0.75 gm./kg. for the tetrachlorophenyl acetate to 2 gm./kg. or higher for most of the other chemicals. The carbonate ester showed no toxicity at 8 gm./kg. The first indication of toxicity was a slightly delayed emergence, which was usually associated with abnormal directional growth of the primary root. The direction of the growth might be horizontal or even almost vertical for several centimeters (Fig. 1). At dosages that were only slightly toxic, this retardation of growth was only temporary, and the seedlings

grew as rapidly as those at non-toxic dosages after normal directional root growth was assumed. Six chemicals, 2,4,5-trichlorophenyl acetate, 2,4,5-tribromophenyl acetate, 2,4,5-trichlorophenyl propionate, tetrachlorophenyl acetate, pentachlorophenyl acetate and o-nitrophenyl acetate, tended to cause a discoloration of the hypocotyl in proximity to the treated seed coat at dosages which approximated those at which abnormal root growth occurred. These blackish discolorations, at times, extended upward in lines on the hypocotyls for several centimeters above the position of the seed coat. The 2,4,6-trichlorophenyl acetate caused no discoloration at toxic dosages, although the seedlings were definitely stunted and the hypocotyls of smaller diameter than those of normal seedlings.

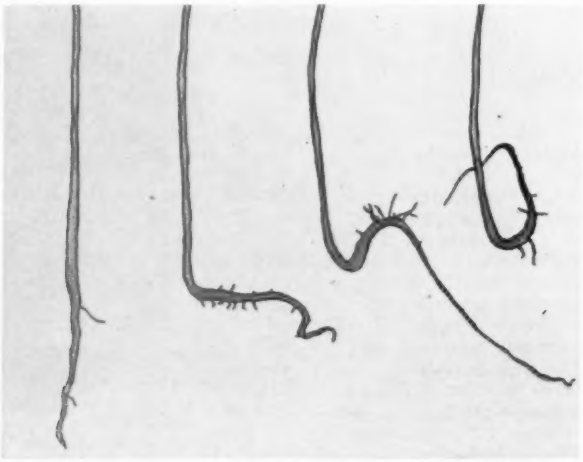

FIG. 1. Abnormal directional root growth of seedlings that developed from seed treated with 2,4,5-trichlorophenol at 3 gm./kg. Normal root at left.

After the initial tests had indicated the fungicidal properties of the four chemicals listed in table 2, further studies were made to ascertain the range of effective dosages and the maximal dosage that would not be toxic to the cotton seedling. The results show almost no safety factor for the 2,4,6-trichlorophenyl acetate, since it was not fully effective at 1.5 gm./kg. and was toxic at 2.0 gm./kg. The tetrachlorophenyl acetate with an effective dosage of about 0.5 gm./kg., with an indication of toxicity at 0.75 gm./kg., and with a definite toxicity at 1.0 gm./kg., had only a slightly greater safety factor. In contrast, the 2,4,5-trichlorophenyl acetate had a safety factor of at least 3, since it was effective in all tests at 0.75 gm./kg. and had no definite toxicity to the seedlings until a dosage of 2.5 to 3.0 gm./kg. was used. This ester varied greatly in its effectiveness at 0.5 gm./kg; the infected seedlings in several tests ranged from 5 to 31 per cent, a variation that might be expected at a dosage approximating the minimal effective dosage. The 2,4,5-tribromophenyl acetate had a somewhat greater safety factor than the corresponding chlorine compound, since it was more

effective than the latter compound at dosages below 0.75 gm./kg; and it became toxic at the same dosage, or 3 gm./kg.

In another test, dosages of 2,4,5-tri- and 2,3,4,6-tetrachlorophenyl acetates at slightly less than the effective dosages, or 0.5 gm. and 0.37 gm., respectively, were applied simultaneously in the same dust to 1 kg. of seed. Only 3 per cent of the hypocotyls of these seedlings were infected which seems to suggest some degree of synergism. The application of 1.5 gm. and 0.75 gm., respectively, in the same dust resulted in the prevention of all seedling infection, but 60 per cent of the roots grew abnormally and 40 per cent of hypocotyls had blackened bases, which would indicate that their toxicity to the host plant is also additive.

The seedlings in the cultures planted to seed treated with the carbonate ester appeared to be injured earlier and more severely by the anthracnose fungus than in those planted to nontreated seed. In order to study this

TABLE 2.—*Comparative percentages of seedlings infected by Colletotrichum gossypii when the seed were treated with the several dosages of the chemicals indicated*[a]

Chemical	Dosage in gm./kg.						
	0.37	0.50	0.75	1.00	1.50	2.00	3.00
2,4,5-trichlorophenyl acetate	.....	15	3	3	4	1	0[b]
2,4,6-trichlorophenyl acetate	.....	57	.....	13	8	2[b]	.....
2,3,4,6-tetrachlorophenyl acetate	10	3	3[b]	3[b]	.....	.....	.....
2,4,5-tribromophenyl acetate	8	7	3	2	4	3	0[b]

[a] The percentages in most instances are means for 3 to 4 tests. For convenience in summarizing the data, in several instances results at a lower dosage were combined with those at the indicated dosage, when the difference in dosage did not exceed 10 per cent.
[b] Slightly toxic to the cotton seedling at this dosage.

more fully, seeds were treated with the dosages indicated in table 3. The results seem to indicate a predisposing effect of the carbonate ester, since after 8 days more seedlings from the seeds treated with the carbonate had visible lesions on the base of the hypocotyl than did the seedlings from nontreated seed. The 8-gm. dosage did not retard emergence.

During these tests a number of other related compounds were tested at a dosage of 2 gm./kg. under similar conditions. These chemicals and the percentages of infected seedlings were as follows: 2,4,5-trichlorophenyl methyl ether, 61; pentachloronitro benzene, 51; bis(5-chloro-2-hydroxyphenyl) methane, 13; bis(5-chloro-2-acetoxyphenyl) methane, 64; and bis(5-chloro-3,5,6-trichlorophenyl) methane, 18. Thus, only the third and fourth chemicals of this series showed any effectiveness, which was not adequate for the treatment of cotton seed, regardless of their use for the mildew-proofing of fabrics (7) and in germicidal soaps (5). The seedlings that developed from the seeds treated with the latter of these two chemicals had chlorotic spots as large as 0.5 cm. in diameter on their cotyledons. This was the only specific pathological effect noted on the tops of the seedlings for any of the 24 chemicals included in these tests.

In order to ascertain whether the results obtained with Seedox[4] in the laboratory might be used to evaluate its effectiveness in field plantings, two lots of Coker 100-WR cotton seed naturally infested by *Colletotrichum gossypii* were used in the field plantings in 1947. Part of the nontreated fuzzy seed of each lot was run through a close-set gin to remove most of the longer seed-coat hairs and obtain the reginned seed. Portions of these two kinds of seeds were treated with the required amount of Seedox to apply the active chemical at rates of 0.5, 1.0, and 1.5 gms. per kg. of seed; other portions were treated with Ceresan-M and Dow-9B, each at dosages of 3 gm./kg., and were included in the plantings for comparisons with Seedox. The treated sub-lots of seed, thus obtained, were germinated in sand culture as described for the previous tests.

The seedlings obtained in the laboratory from the nontreated fuzzy and

TABLE 3.—*Effect of bis(2,4,5-trichlorophenyl) carbonate on the infection of cotton seedlings by Colletotrichum gossypii. Data are based on 96 seeds*

Dosage gm./kg.	Seedling emergence		Seedlings infected	
	Days 6	Days 8	Days 8	Days 10
	No.	*No.*	*No.*	*No.*
0	63	67	27	60
2	70	70	39	68
4	62	62	38	62
8	73	73	44	73

reginned seed of lot A were mostly infected by the anthracnose fungus (Table 4); for lot B a much smaller percentage of the seedlings from the reginned seed were infected than of those from the fuzzy seed. Such differences in the effect of reginning on the relative number of seedlings infected for fuzzy and reginned seed are not unusual. Only small percentages of seedlings were infected in the cultures planted with seed treated with Ceresan-M, Dow-9B, and the two higher dosages of Seedox. The somewhat higher percentages of seedlings that were infected for the fuzzy and reginned seed of lot B treated with the lowest dosage of Seedox indicate that this dosage is too low for effective seed treatment.

In these plantings, there were only three instances in which the percentage of seedlings for a treatment was significantly greater than the percentage for another; namely, Ceresan-M and 2× Seedox relative to Dow-9B on the fuzzy seed of lot A in the April 25 planting and Ceresan-M relative to 1× Seedox on the reginned seed of lot B in the April 18 planting. Thus, the number of significant differences among these treatments are too few to indicate any constant superiority for any of them. The relatively low number of seedlings for the 1× dosage of Seedox on the fuzzy and reginned seed of lot B in the April 18 plantings adds support to the laboratory

[4] Trade name of dust containing 50 per cent of 2,4,5-trichlorophenyl acetate.

results which indicated that this dosage was not great enough to completely destroy the anthracnose fungus on the seed coat.

In contrast to the few significant differences among treatments in the percentage of surviving seedlings, the percentages for the nontreated seed were significantly below those for the best treatment of the same kind of seed in all plantings. The percentages of surviving seedlings for the nontreated reginned seed of lot B were less than the percentage of noninfected seedlings (30 per cent infected) in the laboratory for the same sub-lot of

TABLE 4.—*Comparative results for Seedox, Dow 9B, and Ceresan-M when used to treat portions of two lots of cotton seed*

Type of seed and treatment	Seedlings in sand culture in laboratory at 24°C.				Seedlings surviving in the field (percentage of seeds planted[b])				
	Percentage emerged[a]		Percentage infected by *C. gossypii*		Planting date				
					Apr. 18		Apr. 25		Apr. 26
	Lots								
	A	B	A	B	A	B	A	B	B
Fuzzy seed									
a. Check	65	80	54	79	40	28	39	50	42
b. Ceresan-M	77	92	0	4	56	61	62	68	71
c. Dow 9B	71	84	0	4	59	64	44	63	72
d. Seedox-1 ×[c]	.....	85	.....	17	.....	55	.....	58	72
e. Seedox-2 ×	74	91	3	1	64	67	59	67	75
f. Seedox-3 ×	.....	84	.....	3	.....	70	.....	60	72
Reginned seed									
a. Check	84	80	77	30	37	31	27	39	47
b. Ceresan-M	80	83	1	0	57	60	46	58	68
c. Dow 9B	74	81	0	6	56	47	38	54	68
d. Seedox-1 ×	.....	77	.....	9	.....	43	.....	46	61
e. Seedox-2 ×	73	78	1	0	45	55	38	50	60
f. Seedox-3 ×	.....	73	.....	0	.....	48	.....	43	67
L.S.D.[d]	.....	.....	.....	.....	13	17	16	12	15

[a] Based on the germination of 32 seeds in triplicate.

[b] Based on a quadruplicate planting of approximately 500 seeds.

[c] 1 ×, 2 ×, and 3 × indicate dosages of 0.5, 1.0, and 1.5 gm./kg. of seed, respectively, of 2,4,5-trichlorophenyl acetate, which was applied in a dust containing 50 per cent pyrophyllite as a diluent.

[d] Amount required for significance at 1 per cent level between any two treatments.

seed; while the percentages of surviving seedlings for the other three nontreated sub-lots exceeded in all plantings the percentages of noninfected seedlings in the laboratory. Since these sub-lots were germinated under approximately optimal conditions for seedling infection by the anthracnose fungus, it may be assumed that the seedlings were less severely injured by this fungus in the field than in the laboratory tests, since infected seedlings did not survive in the latter tests. Similar differences between nontreated and treated seed have been obtained in comparisons between field plantings and laboratory tests of previous years. Since these data all indicate a tend-

ency for the seedling infection to be more severe in this particular laboratory technique than in field plantings, this technique may be considered a more rigorous test of the effectiveness of a fungicide in preventing seedling infection by seed-borne mycelia of *Colletotrichum gossypii* than are most field plantings; but it does not necessarily indicate the protection that a given fungicide may afford seedlings against infection by soil-inhabiting pathogens.

DISCUSSION

The effective dosage of 2,4,5-trichlorophenyl acetate for the treatment of cotton seed is almost identical with the dosage of 0.6–1.5 gm. per kilogram of fuzzy seed that Meuli and others (8) found best for the zinc salt of the same trichlorophenol. Their data for the latter salt would indicate a somewhat greater tolerance of the cotton seedling for this chemical than for the acetate (8), since their data show no depression of germination until a dosage of 9 gm./kg. was applied in a 75 per cent dust. In their test, it is questionable, however, whether all of the dust adhered to the seed at dosages above 6 gm./kg. In a field planting of fuzzy cotton seed that had been treated with Zn 2,4,5-trichlorophenate at rates of 1.5, 3.0, and 6.0 gm. per kilogram of seed, the percentages of emerged seedlings were 65, 55, and 37, respectively; which indicates that the zinc salt of 2,4,5-trichlorophenol has about the same toxicity to the cotton seedling as the acetate. Meuli et al. (8) found that the Na salt of 2,4,6-trichlorophenol was somewhat less effective on cotton seed at dosages of 1.0 gm./kg. than the Na salts of the 2,4,5, the tetra-, and pentachlorophenols, which corresponds with their relative effectiveness as esters. In contrast to the results of these tests, which showed a much greater toxicity of the tetrachlorophenol than of pentachlorophenol ester to cotton seedlings, Meuli et al. report greater toxicity to pea seedlings for the pentachlorophenol.

These chlorophenols also have been tested for the control of wood-rotting fungi. Baechler and Bateman (3) found that 0.002 per cent tetra- or pentachlorophenols in nutrient agar inhibited the growth of the fungus studied and that these were among the cheapest of the effective fungicides. These studies were extended by Hatfield (6) to include other chlorophenols. His data show a comparable toxicity for the 2,4,5-tri- and the tetrachlorophenols and a lesser toxicity for the 2,4,6-trichlorophenol. The concentration of pentachlorophenol necessary to inhibit the test fungi was greater than for the other phenols, but the greater insolubility of this chemical made comparisons uncertain. The concentration of the more effective chemicals, the Na salts of 2,4,5-tri- and the tetrachlorophenols, necessary to inhibit the fungi on the agar medium was 0.001–0.002 per cent, or much less than the effective dosages on cotton seed for the 2 4 5-tri- and tetrachlorophenyl acetates, or 0.075 and 0.05 per cent, respectively.

There is no evident correlation between the melting points of the chemicals and their fungicidal activity (Table 1). The 2,4,5-trichlorophenyl acetate has a slightly higher melting point than the 2,3,6- or the 2,4,6-

trichlorophenyl acetate; yet it is the most effective of these acetates. There is, however, a very definite correlation with the position of the chlorine substitutents in the benzene ring. The acid entering into the formation of the ester is also important; the simpler acids, such as formic and acetic, form more effective fungicides than those of higher molecular weight, such as lauric and benzoic acids. The data, however, give no indication of the physical-chemical properties of the phenol derivatives on which their fungicidal activity might be predicated. No irritation was observed while treating the seed with any of the esters that had been prepared from relatively pure chemicals, except for the noticeably pungent formate.

SUMMARY

Cotton seeds naturally infested by *Colletotrichum gossypii* were treated with various substituted phenyl esters and the seeds germinated in sand culture at 24° C. At comparable dosages, the acetate of 2,4,5-trichlorophenol was effective in preventing seedling infection by *C. gossypii*; the formate and propionate esters were slightly less effective; while the carbonate, succinate, and laurate were not effective. The substitution of bromine for chlorine caused relatively little change in effectiveness. The position of the Cl atoms in the benzene ring also influenced fungicidal properties. The 2,3,4,6-tetrachlorophenyl acetate was more toxic to both the fungus and the host plant than the 2,4,5-trichlorophenyl acetate; while the chemicals with the Cl atoms in the 2,4,6, the 2,3,6 and the 2,3,4,5,6 positions were somewhat less effective fungicides. Effective fungicides were not obtained when NO_2 was substituted for Cl in chlorophenyl acetate. Results obtained in field plantings were generally in agreement with the laboratory results and indicated that 2,4,5-trichlorophenyl acetate at a dosage of 1 gm./kg. of seed is a satisfactory treatment for the elimination of the infestation of fuzzy cotton seed by *C. gossypii*.

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CLEMSON, SOUTH CAROLINA

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STRAINS OF MYCOSPHAERELLA FRAGARIAE[1]

A. G. PLAKIDAS

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In the course of many years' work with diseases of strawberries, the writer obtained observational evidence suggestive of the existence of strains of the leaf spot fungus, *Mycosphaerella fragariae* (Tul.) Lindau, varying in their pathogenicity on different varieties of strawberry. Other workers, also, had apparently made similar observations. In private correspondence with the writer, G. M. Darrow pointed out that certain varieties and seedling selections might have a high degree of resistance to this disease in the field in Mississippi and extreme susceptibility in North Carolina, or Maryland, or vice versa. This was suggestive, at least, that different pathogenic strains of the fungus existed in different regions of the country.

Information on whether or not the leaf spot fungus consists of strains differing in their pathogenicity to different varieties of strawberry is of great practical significance. Strawberry breeding projects are in progress in many State experiment stations as well as in the United States Department of Agriculture. Because of the destructiveness of the leaf spot disease in many of the strawberry growing areas of the country, one of the chief objectives of the breeders is to produce varieties that are resistant to this disease. It is, therefore, pertinent to know whether one is dealing with a pathogen which is constant in its reaction or with one composed of strains of varying pathogenicity in respect to different varieties.

It was the object of this study to obtain experimental evidence of whether or not strains of *Mycosphaerella fragariae* exist. It is believed that the data obtained show rather conclusively that this fungus is composed of strains that differ in their pathogenic reaction to different varieties of strawberry.

MATERIALS AND METHODS

Fungus Strains. The following seven strains (isolates) of the leaf spot fungus were used:

S3—isolated from typical leaf spot lesions on Klondike variety of strawberry, from Hammond, Louisiana, 1935.

S18—isolated from atypical lesion (large, brown spots without the grayish center and purple margin) on Klondike, from Hammond, Louisiana, 1935.

S83—isolated from typical lesions on leaves of a seedling strawberry (No. 820) from Corvallis, Oregon, 1938.

[1] A part of this investigation was done at Cornell University, in 1941, under a grant from the General Education Board. The writer wishes to express his grateful appreciation to the Department of Plant Pathology, Cornell University for the use of laboratory and greenhouse facilities and for many courtesies extended to him; and to the Pomology Department, New York (Geneva) Agricultural Experiment Station for supplying many of the strawberry varieties used in these experiments. Other duties during the war, and immediately after, prevented an earlier presentation of the results of this study.

S1388—single-ascospore isolate from dead leaves of wild strawberry (*Fragaria virginiana*) from East Lansing, Michigan, 1939.

S1502—ascospore isolate, culture obtained from Dr. J. B. Demaree, Beltsville, Maryland, 1939.

S1600—isolated from leaves of Geneva Station seedling No. 19747, from Geneva, New York, 1940.

S1610—isolated from leaves of Klondike, from Hammond, Louisiana, 1940.

These seven isolates appeared alike morphologically when grown on the same medium and under the same conditions. All produced *Ramularia* conidia in great profusion. However, no detailed statistical comparison of size and number of conidia or of other characters was made.

Strawberry Varieties. The following ten varieties were used as differential hosts: Klondike, Klonmore, Howard 17 (Premier), Marshall, Dresden, Caledonia, Clermont, U.S.D.A. seedlings Nos. 1021 and 1942, and New Jersey seedling No. 303.

The reactions of the first four varieties to the fungus, under Louisiana conditions, were known from previous experience. The other six were selected in the variety test field of the Pomology department at Geneva. Leaf spot was rather prevalent in this field and it was assumed that all the varieties in it were equally exposed to infection. Therefore, in selecting varieties to be used in the inoculation experiments, those that were free of disease were tentatively considered resistant and those that were badly spotted were considered susceptible. Accordingly, the ten varieties were tentatively grouped, at the beginning of this study, as resistant or susceptible as follows: Klonmore, Howard 17, Dresden, and U.S.D.A. seedlings Nos. 1021 and 1942 were considered resistant; Klondike, Caledonia, Clermont, Marshall, and New Jersey seedling No. 303 were considered susceptible.

Examination of the results of the inoculation experiments (Table 1) will show that with few exceptions these varieties failed to maintain their position in this tentative grouping in response to the different strains of the pathogen. Thus, the choice of these varieties as differential hosts proved a happy one.

Procedure. The fungus was grown on hard (4 per cent) agar in Petri dishes. Beanpod and potato-dextrose agars were used. About 0.5 cc. of a conidial suspension in sterile water was placed in each dish and smeared on the surface of the agar by rotating the plate. In about a week, the surface of the agar was covered with growth which produced conidia in great profusion. When ready to use, sterile water was poured into the dish and, by means of a soft brush, the conidia were brought into suspension.

The inoculum was applied to the plants by means of a glass atomizer attached to a compressed air line equipped with a reducing valve to regu-

late air pressure. Both sides of the leaves were covered with inoculum. The plants were growing well in 7-inch pots on rich garden soil. Two plants of each variety for each strain of the fungus were used in each experiment, and the experiments were repeated at approximately monthly intervals four to six times. Immediately after inoculation, the plants were placed in a large moist chamber of saturated humidity and kept there for 3 days. The temperature of the chamber could be regulated within certain limits, and in these experiments, it was maintained between 75° F. and 80° F. In experiments during the winter months (February–April) at Cornell, the plants were kept in the greenhouse, and daylight was supplemented by 4 hours of artificial illumination. In May and June at Cornell, and in the experiments in Baton Rouge, Louisiana, during the fall and winter, the plants were kept outdoors.

TABLE 1.—*Summary of the results of inoculation experiments with 7 isolates of Mycosphaerella fragariae on 10 varieties of strawberry as differential hosts*

Variety	Isolate						
	S3	S18	S83	S1388	S1502	S1600	S1610
Klondike	++	++	+	+	+	++	++
Klonmore	++	++	T	+	+	+++	+++
Dresden	++	+	T	+	+	+++	++
Caledonia	++	++	+++	+++	+++	+++	++
Clermont	0	0	0	T	+++	+++	0
Howard 17	0	0	0	0	T	T	0
Marshall	++	++	+	+++	T	+++	++
U.S.D.A. Seedling No. 1021	0	0	0	0	0	T	0
U.S.D.A. Seedling No. 1942	+	+	0	0	+	+++	+++
New Jersey Seedling No. 303	+	++	T	++	+++	+++	++

Where infection occurred, lesions began to appear usually between the 12th and 15th day after inoculation, and final records were taken 28 days after inoculation. A rather coarse system of recording degree of infection was used, viz: O = No infection; T = trace of infection, not more than 5 lesions on entire plant; + = light infection, estimated less than 100 lesions per plant; ++ = moderate infection, estimated more than 100 lesions per plant but less than in next class; +++ = heavy infection, estimated several hundred lesions per plant.

The crudeness of this system is acknowledged, but, since all the readings were made by the same individual, it is considered adequate.

DISCUSSION OF RESULTS

It is believed that the results of the inoculation experiments, summarized in table 1, indicate rather conclusively that *Mycosphaerella fragariae* is composed of strains that vary in their pathogenicity toward different varieties of the cultivated strawberry. The reaction of the variety Cler-

mont is particularly clearcut and striking. This variety was heavily spotted from natural infection in the variety test field at Geneva, and it was, therefore, selected as a susceptible host to be used in this study. In the inoculation tests, however, Clermont proved resistant to 5 of the 7 strains of the fungus, but very susceptible to 2, the Beltsville and the Geneva strains. A similar reaction was exhibited by U.S.D.A. seedling No. 1942. It proved resistant, or weakly susceptible, to 5 of the 7 strains, including the Beltsville (S1502) strain, to which Clermont was very susceptible; but it was very susceptible to the Geneva (S1600) strain and one Louisiana strain (S1610).

The Oregon strain (S83) was the least pathogenic, of all the strains used, toward all varieties except Caledonia on which it was very pathogenic.

The Klonmore variety is a cross between Klondike and Blakemore and probably inherited its resistance to leaf spot from the latter, which, in turn, has the highly resistant Howard 17 as one of its parents. In the field in Louisiana, Klonmore generally remains free from leaf spot. Occasionally, it has a limited number of spots, but these usually remain of small size and solitary, and very few conidia are produced on them. However, when inoculated artificially under high humidity conditions, Klonmore is extremely susceptible; the lesions produced are large and typical of the disease, and the pathogen sporulates abundantly on them.

It is not known what factor (or factors) is responsible for the field type of resistance possessed by Klonmore and some other varieties. The results of inoculation certainly do not indicate a chemical type of resistance. Observational evidence indicates that the growth habit of the plant may account for the field type of resistance. Klonmore has an upright type of growth which aids in the quick drying of the leaf surfaces; and it has been shown[2] that the strawberry leaf spot pathogen may cause infection but apparently remain dormant in the tissue without producing external symptoms (lesions) under conditions of low atmospheric humidity. Regardless of its nature, field resistance is of great economic significance. The case of Klonmore is an excellent example of the fact that it is unwise for strawberry breeders to depend on artificial inoculation as a means of eliminating hybrids susceptible to this particular disease. The Klonmore has become the leading commercial variety in Louisiana, occupying at present about 80 per cent of the total strawberry acreage. If artificial inoculation had been used as the criterion, this valuable variety would certainly have been lost.

The reaction of the variety Dresden is probably analogous to that of Klonmore. This variety was completely free from leaf spot in the variety test field at Geneva, and it was, therefore, selected as a resistant host. In the inoculation tests, however, it proved susceptible to all strains of the

[2] Plakidas, A. G. The mode of infection of *Diplocarpon earliana* and *Mycosphaerella fragariae*. Phytopath. **24**: 620–634. 1934.

pathogen including the Geneva strain (S1600) which was present in this field.

Even more difficult to understand is the type of resistance in Howard 17 and U.S.D.A. seedling No. 1021. These two varieties have a degree of resistance approaching immunity both in the field and when inoculated with every one of the strains used in these experiments. This would suggest a chemical type of resistance. However, in a few instances in which a limited number of lesions developed from the inoculation (Table 1), the spots were typical of the disease in size and color, and the pathogen sporulated normally on them. No attempt is made to explain this phenomenon.

Without attempting to explain the nature of resistance, the strawberry varieties used in these experiments may be grouped in three general classes.

I. Those that have resistance both to natural infection under field conditions and to infection by artificial inoculation. Howard 17 and U.S.D.A. Seedling No. 1021 are examples of this class.

II. Those that have a high degree of field resistance but are very susceptible when artificially inoculated. The Klonmore, and probably Dresden, exemplifies this type of resistance.

III. Those that are susceptible both to natural infection in the field and to infection by artificial inoculation. Klondike, Caledonia, and Marshall are good examples of this class.

DEPARTMENT OF PLANT PATHOLOGY,
LOUISIANA AGRICULTURAL EXPERIMENT STATION,
BATON ROUGE, LOUISIANA

THE MOVEMENT OF CROWN-GALL BACTERIA IN ISOLATED STEM FRAGMENTS OF SUNFLOWER[1]

R. S. DE ROPP

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INTRODUCTION

Studies of the movement of crown-gall bacteria in the stems of intact plants were undertaken by Smith, Brown, and McCulloch (5), who concluded that the bacteria were located within the cells of the host plant and were passively transported in tumor strands. Secondary tumors were thought to result from the growth of such strands through the tissues of the stem to sites remote from the primary tumor. Later studies by Riker (2) indicated that crown-gall bacteria first establish themselves in the stem in those water-logged areas which result from wounding. He found that the organisms were capable of moving in the tracheae for distances as great as 9 cm. when cut tomato stems were immersed in suspensions of bacteria. Robinson and Walkden (3) found that, in *Chrysanthemum frutescens* and *Nicotiana affinis*, zoogleal strands of the bacteria could be demonstrated intruding into intercellular spaces and protoxylem vessels, a finding which was later confirmed by Hill (1). Suit and Eardley (6) showed that crown-gall bacteria were capable of moving in the xylem vessels of various plants and that they could remain in the internodes of tomato plants for as long as ten weeks without producing symptoms of crown-gall.

The studies to be described in this paper were undertaken to throw light on the rate of movement of crown-gall bacteria in isolated stem fragments of sunflower cultured *in vitro*. Stem tissue was obtained from four-week-old plants of *Helianthus annuus* L. var. Russian giant. The method of preparation of these fragments and the medium on which they were grown have been described in an earlier paper (4). *Phytomonas tumefaciens*, strain Brown Peach, was employed in all these experiments. The strain was fully virulent, capable of producing tumors both on isolated sunflower stem fragments and on intact plants. Suspensions of the organism were standardized by opacity and viable counts estimated by the colony count technique.

MOVEMENT OF BACTERIA IN FRESHLY ISOLATED STEM FRAGMENTS

Fragments of sunflower stem having an average length of 9 mm. were placed upright on slopes of nutrient agar, only the physiological base being in contact with the medium. A dilute suspension of bacteria was prepared from a 48-hour culture of *Phytomonas tumefaciens* and a small loop-

[1] This research was supported in part by the Committee on Growth of the National Research Council acting for the American Cancer Society.

The writer is indebted to Miss E. Pieczur for technical assistance in connection with this work.

ful of this suspension was placed on the upper cut surface of the isolated stem fragment. Great care was taken to ensure that the drop of bacterial suspension made contact only with the cut surface of the fragment. Colony counts indicated that the number of viable bacteria deposited on the stem by this means ranged from 10 to 100. At different intervals after the application of the suspension, the stem fragments were carefully lifted from the agar and removed. The slopes were then incubated for a week at 25° C. In those instances where the bacteria had passed through the stem fragment, colonies of *P. tumefaciens* developed on the agar at the point on which the base of the fragment formerly rested. The frequency of development of these colonies is shown in table 1.

TABLE 1.—*The movement of Phytomonas tumefaciens through 9-mm. stem fragments of sunflower cultured in vitro*

Interval between application of bacteria and removal of stem fragment from agar	Number of tubes with colonies of *P. tumefaciens*	Number of tubes without colonies of *P. tumefaciens*
None	0	9
4 min.	8	1
30 min.	6	4
1 hr.	3	6
4 hr.	9	1
6 hr.	2	8
8 hr.	6	3
12 hr.	5	5
24 hr.	11	5

The results indicate that crown-gall bacteria were capable of passing through a 9-mm. fragment of sunflower stem in as little as four minutes after being applied to the upper surface of the fragment. Penetration did not occur regularly and there were instances where the bacteria failed to pass through the stem even in 24 hours. This irregularity was probably due to the small number of organisms used. Sections of the material made from 24 to 48 hours after application of the bacteria did not reveal evidence of zoogleal strands within the tissues. It appears from this and from the rapidity of the penetration that these organisms passed through the stem in the tracheae as Riker (2) and Suit and Eardley (6) have suggested. In these isolated stem fragments the transpiration stream mentioned by Suit and Eardley as being largely concerned with the transport of the organisms cannot have played any part. It seems more likely that the mobility of the organisms themselves was responsible for transfer through the stem. According to Riker (2), these organisms are capable of moving at the rate of 1 mm. per minute in distilled water.

A repetition of these experiments using stem fragments placed on the agar on their physiological tops and treated with bacteria on their physiological bases gave similar results to those previously recorded. Evidently, the polarity of the stem fragments did not affect the rate of movement of crown-gall bacteria within them.

THE EFFECT OF CALLUS FORMATION ON PENETRATION OF CROWN-GALL BACTERIA

To determine whether the formation of callus on the cut surface of isolated stem fragments would prevent the penetration of crown-gall organisms, an experiment was set up in which the bacteria were applied to the upper surface of the fragment after it had been cultured *in vitro* for various periods. These stem fragments were not removed from the agar after having been treated with bacteria, and in those instances where the bacteria passed through the fragment, mucilaginous masses of the organisms appeared growing at its base. Suspensions of bacteria were prepared as described in the previous section and the number applied to the upper surface of the stem fragment ranged from 10 to 100 viable organisms. The drop of bacterial suspension was applied in such a way that the metal of the loop did not touch the surface of the stem tissue. Bacteria were applied to the stem fragments immediately after they had been set up and after they had been cultured for three days, one week, and two weeks. The extent to which the bacteria penetrated the stem fragments under these conditions is shown in table 2.

TABLE 2.—*Effect of preliminary culture of isolated stem tips on the penetration of Phytomonas tumefaciens*

Period of culture of stem fragments prior to application of bacteria	Number of stem fragments penetrated by bacteria	Number of stem fragments not penetrated by bacteria
0 days	20	0
3 days	7	12
1 week	0	18
2 weeks	1	18

Evidently after about three days the callus pad which forms on the cut surface of the isolated stem fragment began to present a barrier to the penetration of the crown-gall organism. After a week, this barrier became almost impenetrable. Histological studies revealed that callus formation began to block the open vessels about three days after stem segments were placed on agar. It was presumably this blockage of the vessels that prevented the penetration of the bacteria.

To determine whether the callus that formed at the base of the isolated stem fragment would also act as a barrier to bacterial penetration, this experiment was repeated using stem fragments from which the upper callus pad was removed just prior to the application of the bacteria. The number of stem fragments penetrated under these circumstances is shown in table 3.

Apparently the lower callus pad formed a barrier which was just as effective in preventing the penetration of bacteria as was the upper pad. Bacteria entered the stem fragment and could be isolated from it. They also gave rise to tumors at the cut surface. But owing, presumably, to

the blockage of the vascular elements, the bacteria were unable to escape from the stem fragments onto the agar.

MOVEMENT OF BACTERIA APPLIED TO THE SIDE OF THE STEM FRAGMENT

In this experiment, stem fragments 9 mm. in length were placed on their sides on agar slopes. A small drop of a suspension of crown-gall organisms containing 10–100 bacteria was placed on the upper side of these fragments at an equal distance from both ends immediately after they had been placed on the agar. Great care was taken to avoid contaminating the agar with organisms. Out of a group of 20 stem fragments treated in this way, bacteria appeared on the agar in four instances. Tumors containing crown-gall organisms developed in 11 of the remaining 17 fragments. These tumors were not located at the point of application of the bacteria but grew from the cut surfaces at the ends of the fragments. The structure and point of origin of these tumors will be described elsewhere.

TABLE 3.—*Effect of removal of upper callus pad from stem fragments on the penetration of Phytomonas tumefaciens*

Period of culture of stem fragments	Number of stem fragments penetrated	Number of stem fragments not penetrated
0 days	20	0
3 days	3	14
1 week	0	18
2 weeks	0	20

Here it is relevant to note that crown-gall bacteria applied to the lateral surface of a stem fragment from which the epidermis had been removed were capable of passing along the fragment to its ends and of initiating tumefaction at these points. The route followed by the organisms remains in doubt.

MOVEMENT OF BACTERIA IN INTACT SUNFLOWER STEMS

For comparative purposes, some experiments were carried out on the movement of bacteria in the intact sunflower stem. In one group of five plants, the bacteria were applied to the top of the first internode which had first been decapitated. The organisms were suspended in water and applied with a loop. A dense suspension was used containing in the order of a billion organisms. In a second group of five plants, the organisms were applied at the base of the internode which was cut half way through with a razor, the inoculum being introduced into the cut. These stems were removed one and twenty-four hours after inoculation. After removal of the epidermis, the stems were cut into 5-mm. fragments and each of these fragments was ground up and placed on nutrient agar. The number of fragments from which *Phytomonas tumefaciens* was recovered and the distance of these fragments from the point of inoculation is shown in table 4.

The organisms were evidently capable of moving from 5 to 6 cm. within

the internode in one hour. Their rate of movement and the distance they migrated was approximately equal whether they were applied at the base of the internode or the top. The distance of migration and rate of movement of these organisms in sunflower agree fairly closely with values given by Riker (2) and Suit and Eardley (6) for tomato, bryophyllum, and other plants.

TABLE 4.—*Movement of crown-gall bacteria in intact internodes of sunflower*

Distance of stem fragment from point of inoculation (mm.)	Interval between inoculation and testing			
	1 hour		24 hours	
	Bacteria applied		Bacteria applied	
	At Base	At Top	At Base	At Top
5	5 (5)[a]	5 (5)	3 (3)	4 (4)
10	5 (5)	5 (5)	2 (3)	5 (5)
15	3 (3)	5 (5)	3 (3)	5 (5)
20	3 (3)	3 (3)	2 (3)	5 (5)
25	4 (4)	5 (5)	2 (2)	5 (5)
30	4 (4)	2 (4)	2 (2)	4 (5)
35	4 (4)	2 (4)	3 (3)	4 (5)
40	1 (1)	2 (5)	3 (3)	3 (4)
45	3 (3)	2 (4)	1 (1)	4 (5)
50	1 (1)	1 (4)	3 (3)	2 (3)
55	1 (1)	0 (4)	1 (1)	2 (3)
60		0 (1)		1 (2)
65				1 (1)

[a] The first figure represents the number of stem fragments yielding crown gall bacteria; the figure in parenthesis represents the number of stem fragments tested.

SUMMARY AND CONCLUSIONS

Experiments were undertaken to determine the rate of movement of crown-gall bacteria through isolated fragments of sunflower stem cultured *in vitro*.

A loopful of bacterial suspension containing between 10 and 100 organisms was applied to the upper cut surface of 9-mm. sunflower stem fragments supported on nutrient agar. Bacteria passed through the stem fragment and appeared on the agar within four minutes from the time of application. Polarity of the stem fragment did not affect the rate of movement of the bacteria.

After three days culture *in vitro*, callus formation on the cut surface of the stem fragments hindered the bacteria from passing through the stem. Both the upper and lower calluses proved effectual in this respect.

Bacteria applied to the lateral surface of isolated stem fragments placed on their sides on the medium produced tumors at the end of these fragments.

Bacteria applied to the top or base of intact sunflower internodes travelled about 5 cm. through the stem in the course of one hour. There was no significant difference in the rate of upward or downward movement.

It is concluded that the movement of crown-gall bacteria in isolated

sunflower stem fragments probably takes place through the tracheae and that the motive force is supplied by the bacteria themselves.

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SOME STUDIES OF CURLY TOP OF FLAX

N. J. GIDDINGS[1]

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Experimental infection of flax, *Linum usitatissimum* L., with curly top virus was reported by Severin[2] in 1929. In 1945 Severin and Houston[3] reported the disease from commercial fields in the central San Joaquin Valley of California and gave a brief discussion of the disease in relation to flax growing in that area.

Curly-top diseased flax was received[4] from commercial fields in that same general area in the spring of 1945 and again in 1947 and virus was recovered from these plants.

EXPERIMENTAL WORK

Experiments were begun in the spring of 1945 to determine the relative susceptibility of flax varieties to the different strains of the curly-top virus and to learn whether any of the varieties might be helpful in differentiating the virus strains. Table 1 lists the 37 flax varieties and species which have been tested. The four commercial varieties numbered 19 to 22, namely Acc. 200, Cascade, Highball, and Liral Prince, are "fiber types" and all others are seed types.

All commercial varieties of flax tested were found to be very susceptible to infection by all of the curly-top virus strains available (Fig. 1).

Young plants infected by the highly virulent curly-top virus strains 1, 3, 5, 6, 8, and 9, were dwarfed, distorted, and frequently killed, while those infected by the less virulent strains 2, 4, and 7 showed very little dwarfing and few plants dead, but distinct leaf distortion including enlarged veins, enations, rolling, and twisting as shown in figure 1. Virus strain 10, which is rated as moderately virulent on sugar beets appears to be relatively more virulent toward flax. Of 1687 young flax plants infected by the more highly virulent strains of curly-top virus, under greenhouse conditions, more than 30 per cent were dead in approximately 35 days after inoculation, while in 938 similar plants infected by the less virulent strains the mortality was only 4 per cent in the same length of time, and there was a mortality of 14 per cent among 346 plants inoculated with strain 10.

There were no significant differences in susceptibility to infection among the commercial varieties tested. Varieties 24, Linho das Indias, and 26,

[1] Senior Pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

[2] Severin, H. H. P. Additional host plants of curly top. Hilgardia **3**: 595–637. 1929.

[3] Severin, H. H. P. and Byron R. Houston. Curly top and California aster yellows of flax. Phytopath. **35**: 602–606. 1945.

[4] Collections were made by Dr. Eubanks Carsner of the Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture.

Linho A. R. 81022, had appreciably less injury than others but the seed were very slow in germinating so that the inoculations were made at a different time and the supply of seed was so small that the number of plants involved was limited—12 and 20, respectively. All tests were conducted in a moderately warm greenhouse. Presumably, conditions more nearly simulating the

TABLE 1.—*Flax varieties and species tested for resistance to curly top*

Riverside Number	Name or designation		Source of seed
1[a]	Rio (Argentina)	CI 280	L. G. Goar, El Centro, California
2	Calar do	CI 463	do
3	G. Sel. 1 (India)		do
4	Punjab do	CI 20	do
5	Minerva	CI 1081	J. O. Culbertson, St. Paul, Minn.
6	Rocket	CI 1133	do
7	980 × Redson	CI 1118	do
8	Koto × Redwing	CI 1095	do
9	Sheyenne	CI 1073	do
10	Dakota	CI 1071	do
11	Redwing	CI 320	do
12	Bison	CI 389	do
13	Crystal	CI 982	do
14	Koto	CI 842	do
15	*Linum lewisii* Pursh		Theadore Payne (Seedsman)
16	*L. perenne* L.		Aggler & Musser (Seedsmen)
17	*L. flavum* L.		do
18	*L. grandiflorum* Des F. Var. *rubrum* Hort.		do
19[b]	Acc. 200		Nelson, Oregon
20	Cascade		do
21	Highball		do
22	Liral Prince		do
23	Linho caxias		Brazil
24	Linho das Indias		do
25	Linho Farroupilha		do
26	Linho A. R. 81022		do
27	Linho Reg. 539		do
28	Minn. III–46–1		India
29	Minn. III–46–2		do
30	Minn. III–46–3		do
31	Benvenuto Labrador		Argentina
32	Buck 3		do
33	Entre Rios		do
34	Querondi M. A.		do
35	Buck 114		do
36	Klein II		do
37	La Prevision 18		do

[a] Seed for 1 through 4 were received in 1945; for all others, in 1947.

[b] Numbers 19 to 37 were all received from Dr. J. O. Culbertson and he indicated the original sources of the various lots.

environment under which flax is grown in California would be of greater value in economic evaluation as to curly-top resistance.

Plants infected by the less virulent strains of curly-top virus often show evidences of recovery similar to those reported by Wallace[5] for Turkish

[5] Wallace, J. M. Recovery from and acquired tolerance of curly-top in *Nicotiana tabacum*. Phytopath. 29: 743–749. 1939.

tobacco. Such plants may send out a symptomless branch below the place where most pronounced symptoms appeared or they may merely continue

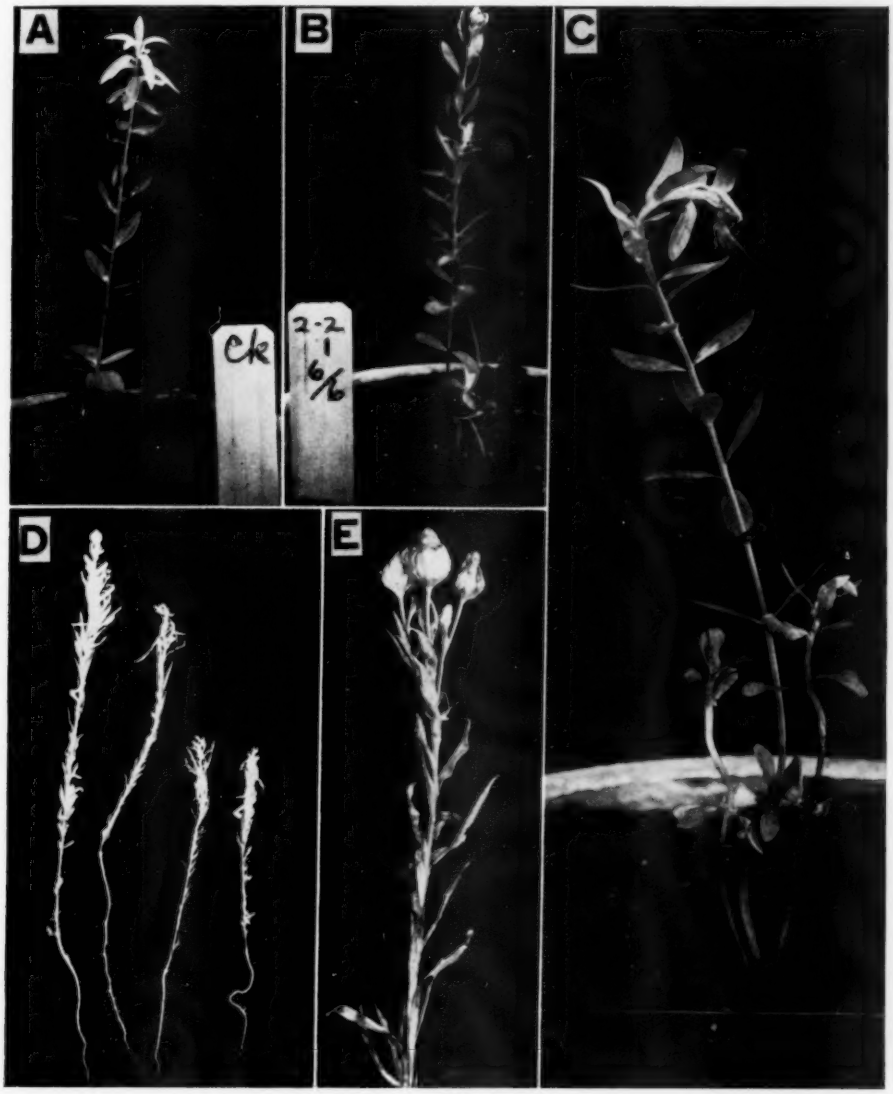

FIG. 1. A. Healthy Rio flax plant on which non-viruliferous leafhoppers had been caged. B. Rio plants with mild symptoms of curly top after inoculation with virus strain 2. C. Curved tips and rugose terminal leaves of Rio flax inoculated with virus strain 8. The enlarged, curved portion of stem is commonly light pink. D. Dwarfed, bleached, and distorted plants infected by curly-top virus, collected from a commercial field of flax in San Joaquin Valley, California. E. Infected plant with distorted leaves and seed pods having papillae on the sepals.

the normal terminal growth with fewer symptoms on the new growth. Symptoms may reappear in the sepals enclosing the seed pod. Four in-

fected plants each having a symptomless branch were tested for the presence of curly-top virus. Virus was recovered from the terminal growth of each plant even though there was very little symptom-bearing tissue included in the cage of non-viruliferous leafhoppers. Virus of a concentration equivalent to that in the terminal growth was found in the symptomless branches of one plant, while another such branch gave evidence of a very low concentration of virus and no virus was obtained from symptomless branches of either of the other two plants.

Four ornamental species of flax, Nos. 15 to 18 in table 1, were also tested. One species, *Linum grandiflorum rubrum*, was fairly susceptible to infection and injury, while the other three, *L. lewisii*, *L. perenne*, and *L. flavum*, were highly resistant. These three resistant species showed no curly-top symptoms and the only evidence of infection was recovery of the virus from infected plants. The available number of plants was limited and *L. lewisii* was tested only with virus strains 1, 2, and 3. Virus was recovered from only one plant and it had been inoculated with strain 2. Plants of *L. perenne* and *L. flavum* were inoculated with virus strains 1, 2, 3, 6, 7, and 10. Tests from these plants revealed that all plants inoculated with virus strain 6 were infected, but no virus was recovered from any of the others.

DISCUSSION AND SUMMARY

The susceptibility of all commercial flax varieties tested indicates the possibility of serious injury during seasons particularly favorable for curly-top infection. Fortunately, the curly-top vector, *Eutettix tenellus* Baker, is likely to be rather inactive during cool fall and winter weather, when the flax is making most of its growth. Early planting or warm, dry weather continuing late into the fall are more favorable for curly-top infection of the young plants and consequently more crop damage.

Comparative studies of flax varieties under field conditions might reveal some differences in susceptibility which would be helpful in breeding for curly-top resistance. Some of the ornamental species have a very high degree of curly-top resistance and this offers some encouragement in the breeding for disease resistance.

There is a possibility that some of the highly resistant ornamental species may be helpful in differentiating curly-top virus strains, since infection of *L. perenne* and *L. flavum* was secured only with strain 6, but further tests are necesssary.

RIVERSIDE, CALIFORNIA

WHEATS IMMUNE FROM SOIL-BORNE MOSAIC VIRUSES IN THE FIELD, SUSCEPTIBLE WHEN INOCULATED MANUALLY[1]

H. H. McKinney

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INTRODUCTION

In conducting the field tests for mosaic resistance in wheat grown in infested soils it has been found that the infestation does not always persist for long periods (2, 4, 9). Sometimes the infectivity fails in one season, whereas, in other cases it may decrease gradually or persist over a period of years with continuous wheat culture. Because of this uncertainty, it has been found advantageous to have the test nurseries located on several infested areas, thereby reducing the chances of losing a season's work.

In all of the wheat-mosaic test nurseries the author has studied east of the Mississippi River, the mosaic-rosette virus (*Marmor tritici* var. *typicum* McK.) (7) and the yellow-mosaic virus (*M. tritici* var. *fulvum* McK.) (7) have been present.[2] When the soil infestations have shown signs of decline, however, it has been observed that the mosaic rosette symptoms in Harvest Queen selection usually fail sooner than do the yellow mosaic symptoms in such varieties as Currell wheat and in Red Winter spelt. Furthermore, at Arlington Farm, Va., when Harvest Queen selection failed to manifest rosette, it failed to manifest any sign of mosaic in most of the plants, even when Red Winter spelt and yellow-mosaic susceptible wheats in adjacent rows had considerable yellow-mosaic mottling. On the other hand, in experiments with these viruses in the greenhouse, Harvest Queen selection had been used regularly for much of the inoculation with the mosaic-rosette virus and with yellow-mosaic virus, since the variety is very susceptible to both when inoculated manually. Furthermore, isolations from plants of resistant varieties manifesting mild signs of mosaic in field plats have yielded the mosaic-rosette virus.

These observations made it appear that Harvest Queen selection and the so-called mosaic-resistant wheats are less susceptible to the yellow-mosaic viruses than to the mosaic-rosette virus when grown in infested soils, and that mosaic resistance in the field is not necessarily a criterion for mosaic resistance following manual inoculation. Accordingly it was

[1] These experiments were conducted at Arlington Farm, Rosslyn, Va., and at the Plant Industry Station, Beltsville, Md. The nursery studies on resistance and immunity in the field were conducted in cooperation with Benj. Koehler, and O. T. Bonnett of the Illinois Agricultural Experiment Station; Ralph M. Caldwell of the Purdue University (Indiana) Agricultural Experiment Station; W. R. Paden of the South Carolina Agricultural Experiment Station, and Coker's Pedigreed Seed Co., Hartsville, S. C.

The author acknowledges the assistance rendered by Matthew Koerner in conducting these tests.

[2] Since preparing this paper, the writer has studied a soil-borne wheat-mosaic virus occurring in the plant breeding plats of the Agricultural Experiment Station at Cornell University, Ithaca, New York. This virus has several characteristics which distinguish it from the viruses discussed in this paper.

decided to make manual-inoculation studies on some of the wheat lines that had been selected for high mosaic resistance, or were immune from mosaic when grown in the field in soils having high levels of infestation. The results of these tests have been summarized in an abstract (9).

It should be pointed out that the situation is quite different with the rosette expression. Here, there has been complete correlation between the field-grown plants and the manually inoculated plants. The great majority of wheat varieties are completely immune from rosette, apparently under all conditions of culture and of inoculation. However, most varieties are susceptible to the mosaic reaction induced by the mosaic-rosette virus.

PLANT MATERIALS

Harvest Queen selection was selected by the writer from the mass of Salzer's Prizetaker (Harvest Queen C.I. 5314). It is uniformly susceptible to mosaic-rosette in the field and also in greenhouse tests when inoculation is by manual methods.

Michigan Amber (Ind. Sel. 29–1–1–1) is uniformly susceptible to the green and the yellow mosaics, but it is immune from rosette in the field and in the greenhouse tests.

The mosaic-resistant wheats listed in table 1 develop no signs of mosaic or only weak to moderate mottling in few to many plants, depending on the season. They are uniformly immune from rosette in the field, and when inoculated by manual methods.

The field-immune lines have shown no signs of mosaic or rosette in the field, except one case to be noted. Ill. Sel. 36–686 gave no signs during about 10 years' testing on infested soil in Illinois. In 1945, however, one yellow-mosaic plant was found in two 16-foot rows in the wheat-mosaic nursery at Clemson, South Carolina. Adjacent rows of susceptible wheats had mosaic in a high percentage of the plants. It is possible that an unusual strain of virus infected this plant, but it seems more likely that the plant represented an admixture, possibly a volunteer.

The winter barley lines expressed no signs of mosaic or rosette when tested one season in the wheat-mosaic nurseries. Further information on the plant materials is given in table 1.

VIRUS COLLECTIONS

Isolates of mosaic-rosette virus (*Marmor tritici* var. *typicum*) (7) and of the yellow-mosaic virus (*M. tritici* var. *fulvum*) (7) were used. The several isolates of mosaic-rosette virus were indistinguishable, but several isolates of the yellow-mosaic virus produced consistently different symptoms on Michigan Amber wheat. All of the latter induce yellow-green to yellow markings on the foliage of Michigan Amber, and all stunt the plants. The isolates from La Porte, Indiana, and Granite City, Illinois (Table 3) are less severe than the others. The isolate from LaFayette, Indiana

(Table 4) induces extreme rolling and distortion of the leaves during their early stages of development. The isolate from Clemson, South Carolina

TABLE 1.—*Information concerning the winter wheats and winter barleys used*

Variety or selection	C.I. No.	Source of seed	Reaction to mosaic in infested soil in the field
Wheat:			
Michigan Amber 29–1–1–1 (Mildew resistant)	11770	R. M. Caldwell	Immune from rosette; very susceptible to light-green mosaic with mosaic-rosette virus; yellow mosaic with yellow-mosaic viruses.
Harvest Queen selection		H. H. McKinney	Very susceptible to rosette and light-green mosaic with mosaic-rosette virus; very resistant to or immune from yellow-mosaic viruses.
Arlando	10069	W. J. Sando	Immune from rosette; resistant to or immune from yellow mosaics; mildly susceptible to green mosaics.
Harvest Queen sel. 32–43		H. H. McKinney	Do
Ill. sel. 37–D216 from Duffy		O. T. Bonnett and B. Koehler	Do
Ill. sel. 37–D382 Fulcaster × Minturki		Do	Do
Ill. sel. 37–D544 (Minturki × Fulcaster) × Fulcaster		Do	Do
Ill. sel. 37–1146 (Kanred × Red Rock sel. H–93–2) × (Malakof × Mich. Amber)		Do	Do
Wabash	11384	R. M. Caldwell	Do
Ill. sel. 36–686 from (Gladden × Minturki) × Gladden		Bonnett and Koehler	Immune from the mosaics and rosette.
Ind. sel. 11512 from Trumbull × Fultz	12220	R. M. Caldwell and L. E. Compton	Do
Supreza × (Redhart Strain 5)		Coker's Ped. Seed Co.	Do
Barley:			
Purdue 21 (Mass sel. from Tenn. winter)	4581	Bonnett and Koehler	Do
Purdue 25 Comfort × Purdue 21		Do	Do
Purdue 28 Velvet × Purdue Acc. 1101		Do	Do
Smooth Awn 86	6268	J. W. Taylor	Do

(Table 5) does not induce leaf rolling, but it stunts the plants badly, usually causing early death in most of the population. The destruction of the plastid pigments in Michigan Amber is greatest with this isolate.

In the tests with the field-immune wheat, Trumbull × Fultz sel. 11512 (C.I. 12220), from R. M. Caldwell and L. E. Compton, the writer collected a field composite of virus from mosaic Michigan Amber plants growing immediately adjacent to each end of the nursery row of the field-immune line. This was to assure, so far as possible, the testing of this wheat against the virus population to which it had been subjected in the nursery near LaFayette, Ind. As this series of tests progressed, care was taken to get virus from the leaves of all the diseased plants of a population supplying inoculum for a subsequent test.

METHODS

All seedlings were cultured in steamed soil in 6-inch earthen pots in a cool greenhouse during the winter or in a special culture chamber during the hot months. The temperature was regulated near 60° to 65° F. In the chambers, the daily photoperiod was of 8 hours' duration.

Seedlings were inoculated when they reached the three-leaf stage. In most of these tests, the leaves of the seedlings were inoculated by drawing them between the thumb and fingers moistened with the infective juice to which 600-grain carborundum powder had been added. The leaves were then rinsed with water. When the roots or the underground leaf sheaths were inoculated, the seedlings were carefully removed from the soil or the sand, the roots were washed, and inoculation proceeded in the same manner outlined for the leaves, but without the rinsing. In one test in 1934, the inoculum was worked into the crown tissues by means of a fine needle, and the roots were bathed in the virus extract. Pouring fresh virus extracts into the soil around the seedlings failed to induce mosaic. According to Allen (1) this method of inoculation induces big vein disease (a soil-borne virus disease) in a high percentage of lettuce plants.

Inoculum was prepared from fresh mosaic leaves from moderately young wheat plants. The tissue was clipped and then thoroughly pulped with sand in a mortar, with sufficient sterile water added to make a five-fold dilution of the plant juice. The resulting fluid was passed through double surgical gauze to remove the coarser fragments of tissue and sand. The carborundum powder was added to the liquid, and the inoculation procedure was started immediately. All plants inoculated with a given virus on a given day were inoculated from the same batch of virus extract, therefore, the varietal comparisons should be confined to a given batch of inoculum.

No method of manual inoculation has yet been devised that will produce infection with the viruses under study in all of the inoculated plants in every test, even though the host is known to be homozygous for susceptibility. Throughout the investigations on these wheat viruses, many thousands of wheat seedlings have been inoculated by the leaf-wiping method, and by the pricking and root-wiping method, and it has been found

that the former usually induces the higher percentage of infection. In addition, it is much simpler and faster than the pricking and root-wiping method used in the earlier work (2, 4). Furthermore, since using this leaf-wiping method, and by giving special attention to the use of virus from moderately young succulent plants, the cultures of these viruses have shown no signs of becoming inactive, as when the earlier methods were employed (7).

RESULTS

The results in tables 2 to 6 show that field resistance to and field immunity from mosaic in wheats is not reflected in tests where the inocula-

TABLE 2.—*Results from inoculations with Marmor tritici var. typicum (wheat mosaic-rosette virus) on winter wheats and winter barleys cultured in greenhouses or in low-temperature chambers. Virus from plants grown in soil collected near LaFayette, Indiana, spring of 1943. Inoculation was by the leaf-wiping method*

Variety or selection	Date inoculated	Seedlings		
		Inoculated	Infected[a]	
		Number	*Number*	*Per cent*
Wheat:				
Michigan Amber (S)[b]	Feb. 5, 1945	29	26	89.65
Arlando (R)	Do	20	20	100.00
Ill. sel. 37–D216 (R)	Do	26	24	92.31
Ill. sel. 37–D382 (R)	Do	24	24	100.00
Ill. sel. 37–D544 (R)	Do	33	29	87.88
Ill. sel. 37–1146 (R)	Do	28	25	89.28
Michigan Amber (S)	May 19, 1945	63	25	39.68
Ill. sel. 36–686 (I)	Do	51	26	50.98
Barley:				
Purdue 21 (I)	Feb. 5, 1945	32	5	15.62
Purdue 25 Hybrid (I)	Do	22	1	4.54
Purdue 28 Hybrid (I)	Do	18	0	0
Smooth Awn 86 (I)	Do	29	0	0

[a] In all varieties and lines, the symptoms consisted of light-green mosaic mottling, but they tended to be strongest in Ill. sel. 36–686, the only field-immune wheat in this series of tests.

[b] Varietal comparisons should be confined to the separate inoculation test dates, as the lots of inoculum used in the different tests could not be standardized for virus concentration. Letters in parentheses signify the reaction of a variety in the field tests as follows: (R) Resistant, (S) Susceptible, (I) Immune.

tion is by manual methods. With all of the virus collections used, the mosaic symptoms were as strong in wheats that are resistant or immune in the field as they were in the susceptible controls. In one case (Table 2), a field-immune wheat gave a stronger mosaic reaction than obtained in the susceptible control.

When grown in the infested nurseries, all of the resistant lines and varieties manifest mild to moderate mosaic symptoms in few to many plants during some seasons, whereas, they may show no signs during other seasons, even though the susceptible controls have much mosaic, much of it being yellow mosaic. In the present tests with manual inoculation, only the

mild mosaic developed when the mosaic-rosette virus was used (Table 2). When the yellow-mosaic virus isolates were used, the field-resistant lines developed yellow mosaic, just as in the field-susceptible Michigan Amber controls (Tables 3 to 5). The degrees of stunting and leaf malformation, however, varied among the several wheat lines.

When comparisons are based on the percentage of the inoculated seedlings developing mosaic after the first inoculation, there is little indication of varietal difference in resistance. In tables 2 to 5 it will be observed that most of the tests show the higher percentages of mosaic plants in the field-resistant and field-immune lines. This may be accounted for by the

TABLE 3.—*Results from inoculations with Marmor tritici var. fulvum (Prairie wheat yellow-mosaic virus) on winter wheat cultured in greenhouses. Virus isolate used in 1934, collected near Granite City, Illinois, and isolate used in 1946, collected near La Porte, Indiana. Inoculation was by the leaf-wiping method, except as indicated*

Variety or selection	Date inoculated	Virus dilution	Seedlings		
			Inoculated	Infected[a]	
			Number	*Number*	*Per cent*
Harvest Queen sel. (R)[c]	Mar. 13, 1934	5×	36	23	63.89[b]
Harvest Queen sel. 32–43 (R)	Do	Do	24	19	79.17[b]
Michigan Amber (S)	Apr. 5, 1946	Do	9	7	77.78
Ill. sel. 36–686 (I)	Do	Do	9	7	77.78
Michigan Amber (S)	Do	250×	31	9	29.03
Ill. sel. 36–686 (I)	Do	Do	31	9	29.03
Michigan Amber (S)	Feb. 10, 1947	5×	48	48	100.00
Ind. sel. 11512 (I)	Do	Do	48	47	97.92

[a] In all varieties and lines the symptoms consisted of mild to severe yellow mosaic mottling.

[b] Inoculation was accomplished by removing the seedlings from the soil, dipping the roots and crowns in fresh inoculum, and pricking the inoculum into the crown and leaf sheaths with a fine needle.

[c] Varietal comparisons should be confined to the separate inoculation test dates, as the lots of inoculum used in the different tests could not be standardized for virus concentration. Letters in parentheses signify the reaction of a variety in the field tests as follows: (R) Resistant, (S) Susceptible, (I) Immune.

fact that there is a small proportion of Michigan Amber susceptible controls in these tests. In table 6, where more Michigan Amber controls were tested, the differences are more in favor of the resistant and immune lines, but they are slight when compared with the differences that obtain in the field. In all of the tests (Tables 2 to 6), there is a total of 32 comparisons that may be made between the susceptible control and the resistant and immune wheats. Of these comparisons, 14 show more, and 14 show fewer mosaic plants among the field-resistant and field-immune wheats. Four of the comparisons are equal.

With the composite collection of virus (Table 6), there are 7 comparisons, 2 of which show more, and 5 show fewer mosaic plants among the field-immune lines. Taking the totals from all the tests in table 6, Michigan Amber susceptible control developed mosaic in 42.71 per cent of the

seedlings after the first inoculation, whereas, the field-immune wheats developed mosaic in 33.85 per cent of the seedlings. This drop of 8.86 per

TABLE 4.—*Results from inoculations with the leaf-distorting strain of Marmor tritici var. fulvum (Prairie wheat yellow-mosaic virus) on winter wheats and winter barleys cultured in greenhouses. Virus from plants grown in soil collected near Lafayette, Ind., in 1939. Inoculation was by the leaf-wiping method, except as indicated in the footnote*[d]

Variety or selection	Date of test	Virus dilution	Seedlings					
			Inoculated	Infected[a]		Re-inoculated	Infected	
			No.	*No.*	*Pct.*	*No.*	*No.*	*Pct.*
Wheat:	Jan. 11,							
Michigan Amber (S)[b]	1945	5×	27	16	59.26	11	11	100.00
Arlando (R)	Do	Do	20	19	95.00	1	1	100.00
Ill. sel. 37–D216 (R)	Do	Do	26	19	73.08	7	7	100.00
Ill. sel. 37–D382 (R)	Do	Do	27	26	96.30	1[c]	0	96.30
Ill. sel. 37–D544 (R)	Do	Do	29	23	79.31	6	6	100.00
Ill. sel. 37–1146 (R)	Do	Do	28	28	100.00	.....	.....	.....
	Feb. 6,							
Michigan Amber (S)	1946	Do	50[d]	0	0.00	.....	.....	.....
Ill. sel. 36–686 (I)	Do	Do	50[d]	0	0.00	.....	.....	.....
	Feb. 25,							
Michigan Amber (S)	1946	Do	43	25	58.14	.....	.....	.....
Wabash (R)	Do	Do	50	19	38.00	.....	.....	.....
	Apr. 5,							
Michigan Amber (S)	1946	Do	9	9	100.00	.....	.....	.....
Ill. sel. 36–686 (I)	Do	Do	8	6	75.00	.....	.....	.....
Michigan Amber (S)	Do	250×	36	21	58.33	.....	.....	.....
Ill. sel. 36–686 (I)	Do	Do	28	13	46.43	.....	.....	.....
	Jan. 17,							
Michigan Amber (S)	1947	50×	30	30	100.00	.....	.....	.....
Ill. sel. 36–686 (I)	Do	Do	56	56	100.00	.....	.....	.....
	Feb. 10,							
Michigan Amber (S)	1947	5×	48	42	87.50	.....	.....	.....
Ind. sel. 11512 (I)	Do	Do	48	44	91.67	.....	.....	.....
	Feb. 25,							
Michigan Amber (S)	1947	Do	24	24	100.00	.....	.....	.....
Ill. sel. 37–D382 (R)	Do	Do	26	24	92.31	2	2	100.00
Barley:	Jan. 11,							
Purdue 21 (I)	1945	Do	28	1	3.57	27	1	7.14
Purdue 25 (I)	Do	Do	21	0	0.00	21	0	0.00
Purdue 28 (I)	Do	Do	28	1	3.57	27	0	3.57
Smooth Awn (I)	Do	Do	29	1	3.45	28	6	24.14

[a] In all varieties and lines, the symptoms consisted of mild to severe yellow-mosaic mottling.

[b] Varietal comparisons should be confined to the separate inoculation test dates, as the lots of inoculum used in the different tests could not be standardized for virus concentration. Letters in parentheses signify the reaction of a variety in the field tests as follows: (R) Resistant, (S) Susceptible, (I) Immune.

[c] This plant was inoculated a third time and failed to become infected. It was not inoculated again as resistance tends to increase with age, but it was isolated from other wheat plants, and allowed to set seed. This seed was sown, and the 26 seedlings were inoculated Feb. 25, 1947, with the results shown in the last item under wheat in this table.

[d] Inoculation was accomplished by removing the seedlings from the soil, and wiping the underground portion of the first leaf sheath and the roots with fresh virus extract and carborundum powder.

cent in the incidence of mosaic is not very great when we take into account that these wheats have been immune in the fields.

It was not practicable to reinoculate all of the seedlings that escaped infection after the first inoculation. This was done, however, in a few tests shown in tables 4 and 6. In some of these tests, all of the escapes developed mosaic after the second inoculation. In table 4, one plant of Ill. sel 37–D382 failed to show mosaic after being inoculated three times. However, when the progeny (26 seedlings) from this plant was inoculated, 24 seedlings developed yellow mosaic from the first inoculation and the remaining two after a second inoculation. In table 6 it will be noted that a fairly large percentage of the seedlings remained mosaic-free after the second inoculation.

When the composite virus collection was used (Table 6), the symptoms consisted of a mixture of green and of yellow mosaic, and as the tests pro-

TABLE 5.—*Results from inoculations with Marmor tritici var. fulvum (Prairie wheat yellow-mosaic virus) on winter wheat cultured in greenhouses. Virus collected near Clemson, South Carolina. Inoculation was by the leaf-wiping method*

Variety or selection	Date of test	Virus dilution	Seedlings		
			Inoculated	Infected[a]	
			Number	*Number*	*Per cent*
Michigan Amber (S)[b]	Apr. 5, 1946	5×	10	3	30.00
Ill. sel. 36–686 (I)	Do	Do	9	3	33.33
Michigan Amber (S)	Do	250×	33	7	21.21
Ill. sel. 36–686 (I)	Do	Do	33	7	21.21
Michigan Amber (S)	Dec. 30, 1946	50×	48	47	97.92
Supreza × (Redhart Str. 5) (I)	Do	Do	45	43	95.55
Michigan Amber (S)	Jan. 17, 1947	Do	30	30	100.00
Supreza × (Redhart Str. 5) (I)	Do	Do	51	49	96.08

[a] In all varieties and lines the symptoms consisted of moderate to severe yellow-mosaic mottling.

[b] Varietal comparisons should be confined to the separate inoculation test dates, as the lots of inoculum used in the different tests could not be standardized for virus concentration. Letters in parentheses signify the reaction of a variety in the field tests as follows: (R) Resistant, (S) Susceptible, (I) Immune.

gressed, the proportion of green mosaic tended to increase very slightly, suggesting that unilateral interference was operating very weakly in the culture. All observations to date support the view that if unilateral interference does occur among the viruses that have been isolated from wheat plants that contracted mosaic by way of the soil, it is not a striking phenomenon (3, 7). Weak unilateral interference (antagonism) was reported by the writer (5) in three mutants from tobacco-mosaic virus, but in these cases, each of the two yellow-mosaic mutants dominated the mild-green mosaic mutant.

Facilities have not permitted a thorough study of virus isolates from the mild-mosaic wheat plants that occur in the resistant lines in the field, but the few isolations that have been made indicate that some of these plants are infected with the mosaic-rosette virus. Also, it appears that

non-rosetting green-mosaic viruses are present in some areas, i.e., viruses that will not induce the rosette reaction in Harvest Queen selection.

In tables 2 and 4 it will be noted that very few plants of the field-immune barley lines developed mosaic in the manual-inoculation tests.

TABLE 6.—*Results from inoculations with a field composite collection of strains of Marmor tritici on winter wheats cultured in greenhouses and low-temperature culture chambers. The virus culture was isolated from mosaic Michigan Amber plants growing near Lafayette, Ind., in nursery rows adjacent to each end of a row of Ind. sel. 11512 that developed no mosaic in the field. Inoculation was by the leaf-wiping method, except as indicated in the footnotes*

Variety or selection	Date of test	Virus dilution	Seedlings					
			Inoculated	Infected[a]		Re-inoculated	Infected	
			No.	*No.*	*Pct.*	*No.*	*No.*	*Pct.*
Michigan Amber (S)[b]	June 21, 1946	5×	46	30	65.22	.....	.....	.....
Ind. sel. 11512 (I)	Do	Do	50	33	66.00	.....	.....	.....
Michigan Amber (S)	Aug. 15, 1946	Do	80	16	20.00	.....	.....	.....
Ind. sel. 11512 (I)	Do	Do	80	10	12.50	.....	.....	.....
Michigan Amber (S)	Oct. 21, 1946	Do	40	21	52.50	.....	.....	.....
Ind. sel. 11512 (I)	Do	Do	40	12	30.00	.....	.....	.....
Michigan Amber (S)	Dec. 2, 1946	50×	40	13	32.50	27	16	72.50
Ind. sel. 11512 (I)	Do	Do	40	3	7.50	37	10	32.50
Michigan Amber (S)	Dec. 30, 1946	Do	58	30	51.72	28	6	62.07
Ind. sel. 11512 (I)	Do	Do	61	40	65.57	21	7	77.05
Ill. sel. 36–686 (I)	Do	Do	66	31	46.97	35	9	60.61
Michigan Amber (S)	Jan. 17, 1947	Do	24	7	29.17	.....	.....	.....
Ind. sel. 11512 (I)	Do	Do	59	5	8.47	.....	.....	.....
Michigan Amber (S)	Jan. 28, 1947	5×	53	18	33.96	.....	.....	.....
Do	Do	Do	40[c]	3	7.50	.....	.....	.....
Do	Do	Do	40[d]	2	5.00	.....	.....	.....
Ind. sel. 11512 (I)	Do	Do	40[c]	1	2.50	.....	.....	.....
Do	Do	Do	40[d]	0	0.00	.....	.....	.....

[a] In each test, the mosaic mottling was essentially of the same degree in the field-immune wheat as in Michigan Amber, the field-susceptible wheat.

[b] Varietal comparisons should be confined to the separate inoculation test dates, as the lots of inoculum used in the different tests could not be standardized for virus concentration. Letters in parentheses signify the reaction of a variety in the field tests as follows: (S) Susceptible, (I) Immune.

[c] Inoculation was accomplished by removing seedlings from the soil and wiping the underground portion of the first leaf sheath with fresh virus extract and carborundum powder.

[d] Inoculation as above, except that the roots only were wiped.

Comparatively few varieties of barley have been tested in the mosaic fields, but the tests that have been conducted suggest that barley tends to be more resistant than wheat. However, Nakano Wase barley is extremely susceptible to mosaic in the field.

CONCLUSIONS

When grown in virus-infested soils, it appears that the field-resistant wheats may be resistant in varying degrees to one or more of the green mosaics, and immune, in most cases, from the yellow mosaics. Whereas, the field-immune wheats appear to be immune from both green and yellow mosaics. The breakdown of immunity from mosaic following inoculations by manual methods cannot be explained at present. It is possible that a more delicate method of manual inoculation might reflect the same varietal differences in mosaic susceptibility as obtained in the field. This does not seem to be a very promising approach, however, at present. It may be that none of the infested soils encountered thus far contain sufficient amounts of active virus to induce yellow mosaics in the field-immune wheats. If it were possible to increase virus activity or concentration in the soil, this point could be tested, but all attempts to infest soil by adding mosaic leaf tissue or fresh virus extracts have failed. The field differences in susceptibility might be reflected if the manual inoculations were made on older plants which are more resistant than the seedlings and young plants. It is possible that immunity in the field is against a vector of some sort, rather than directly against virus. If this is the case, more than one vector may be involved, and there may be a hitherto unsuspected, strong interference between the yellow-mosaic and the green-mosaic viruses when present in certain genotypes of wheats. Otherwise, it is difficult to understand how one group of wheats can be susceptible to green mosaics and immune from yellow mosaics, whereas another group growing in the same immediate area in a field will be immune from both green mosaic and yellow mosaic.

It seems likely that the range of reaction shown among the virus isolates used in these studies will be extended as new isolates are studied, especially isolates from plants out of the field-resistant group. It is already known that the mosaic-rosette virus induces a moderately yellow mosaic in Red Winter spelt (3, 4), whereas, in all of the wheats tested against this virus in the present studies, only light-green mosaics occurred. Whether a study of new isolates will shed further light on the problems covered by the present tests, is a question. The wheats and the virus isolates used in the tests were chosen at random, yet all of the wheats reacted alike, within narrow limits, rosette excepted, when inoculated with any one of the several virus isolates used.

With the tobacco-mosaic virus it is possible to make rapid progress in the resistance studies with tobacco (6, 8) and other crops when manual methods of inoculation are used, but it is evident from the results presented, that this is not true in the case of the soil-borne mosaic viruses in wheat. Here the routine testing and the genetic studies must be done in the infested soil, except in the case of the rosette reaction, which can be studied when the inoculations are done manually. With the mosaic reactions, precise genetic studies will be difficult, even when closely coordi-

nated with studies on the several isolates which make up the virus population of the test soil, because there is no way at present to test the virus isolates separately by nature's method of inoculation.

PLANT INDUSTRY STATION,
BELTSVILLE, MARYLAND.

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DIP AND GAS TREATMENTS FOR THE REDUCTION OF POST-HARVEST DECAY IN TEXAS LEMONS

A. Lloyd Ryall[1] and G. H. Godfrey[2]

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A previous publication[3] described the effects of wrappers impregnated with diphenyl, chemical dips, and stem swab treatments for the control of decay in lemons after harvest. Many chemicals used as dips were fairly effective in reducing Diplodia and Penicillium rots. However, none of the materials that were considered to be safe from the standpoint of fruit injury and chemical residues were sufficiently fungicidal to ensure that the fruit would be commercially acceptable after the usual transit and marketing period.

A gaseous mixture of nitrogen trichloride and air has been reported by Klotz[4] to be effective in controlling *Penicillium italicum* (blue mold) and *P. digitatum* (green mold) decay of Washington Navel and Valencia oranges in California. Its effectiveness for control of stem-end decay (*Diplodia natalensis*) was not known.

As Texas lemons are commonly placed in a coloring (degreening) room for treatment with ethylene gas for one day to 6 or 7 days, depending upon harvest maturity, it was felt that the coloring room would be the logical place to treat the fruit with a fungicidal gas. Since certain chemicals had been demonstrated to be at least partially effective as dips before coloring, the experiments were set up in such a way that results could be obtained on dips alone, on gas alone, and on a combination of the dip and gas treatments.

METHODS

Eureka lemons were harvested early in October, late in October, and in December and January. As soon as the fruit was brought in from the field, composite samples of 200 fruits each were selected by taking 10 to 12 fruits of normal size and appearance from each of 17 to 20 field boxes. Injured or overripe fruits were discarded. Dipping was done as soon as sampling was completed, then all were placed in the coloring rooms. Fruits in one room received the regular commercial coloring treatment with ethylene gas. Fruits in the other received ethylene interrupted at intervals for the introduction of the mixture of NCl_3 and air. The first treatment with NCl_3 always was applied in the evening of the day the fruit was harvested and

[1] Horticulturist, Division of Fruit and Vegetable Crops & Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

[2] Plant Pathologist, Rio Grande Valley Substation, Texas Agricultural Experiment Station.

[3] Godfrey, G. H., and A. L. Ryall. Control of transit and storage decay in Texas lemons. Texas Agr. Expt. Sta. Bul. (in press)

[4] Klotz, L. J. Nitrogen trichloride and other gases as fungicides. Hilgardia 10: 27–52. 1936.

within a few hours of the time the fruit was placed in the coloring room. Four hours was the usual duration of treatment and the concentration of NCl_3 varied from 0.003 to 0.04 p.p.m. Additional treatments with NCl_3 were made at intervals of 24 to 48 hours.

The lemons were held in the coloring room for 8 days in early October but only 2 days in January, the time depending upon the degree of coloration when the fruits were harvested. At the completion of coloring they were removed from the room, examined for decay, wrapped in regular tissue wrappers, and packed in standard boxes which were held in a non-refrigerated storeroom in the warehouse at temperatures that varied from 75° to 85° F. in October and from 50° to 75° F. in January.

Each lot was inspected for decay one week after packing, after which it was repacked and held for an additional week to ten days. The final inspection was made 20 to 22 days after harvest.

RESULTS

In lemons harvested early in October stem-end rot was responsible for all of the losses during the holding period (Fig. 1,A). The lot that received no dip after harvest and no nitrogen trichloride during degreening averaged over 50 per cent decay 1 week after packing and 70 per cent decay 2 weeks after packing. The lot that received no dip but was treated with a very low concentration (0.003 p.p.m.) of NCl_3 in the coloring room had very little decay 1 week after packing but 13.5 per cent in the second week. A dip with the quaternary ammonium compound "Adbac" had very little effectiveness at the concentration used, but when this dip was followed by treatment with NCl_3 the stem-end rot was only 2 per cent 2 weeks after packing.

In lemons picked in late October, stem-end rot was responsible for all of the decay found during the holding period (Fig. 1,B). One week after packing, the untreated lot of Eureka lemons had 64 per cent decay and the similar lot of Meyer lemons, a little less than 30 per cent. A slightly higher concentration of NCl_3 (0.005 p.p.m. as Cl_2) than that used in the first test gave only fair control when used on non-dipped fruit. Neither the Adbac dip nor the thiourea dip, used without subsequent NCl_3 treatment, was adequate for control although both substantially reduced decay as compared with no dip check.

By the time the third experimental lot was picked (Dec. 9) the blue and green molds were becoming a factor in total decay, although stem-end rot still predominated, particularly in the non-gassed lots (Fig. 1,C). In this test the concentration of NCl_3 was increased to 0.03 p.p.m. and the results indicate that the gas was more effective at this strength than at lower concentration. The untreated check had 22 per cent stem-end rot and 4 per cent blue and green mold rot, or a total of 26 per cent decay, 17 days after packing, whereas the non-dipped but NCl_3 treated fruit had 2 per cent stem-end rot and 1.5 per cent green mold rot, or a total of 3.5 per cent decay, after the same period.

With lemons harvested early in January, stem-end rot was of minor importance and the predominating decay was green mold (Fig. 1,D). The untreated check lot had over 29 per cent blue and green mold rot and less

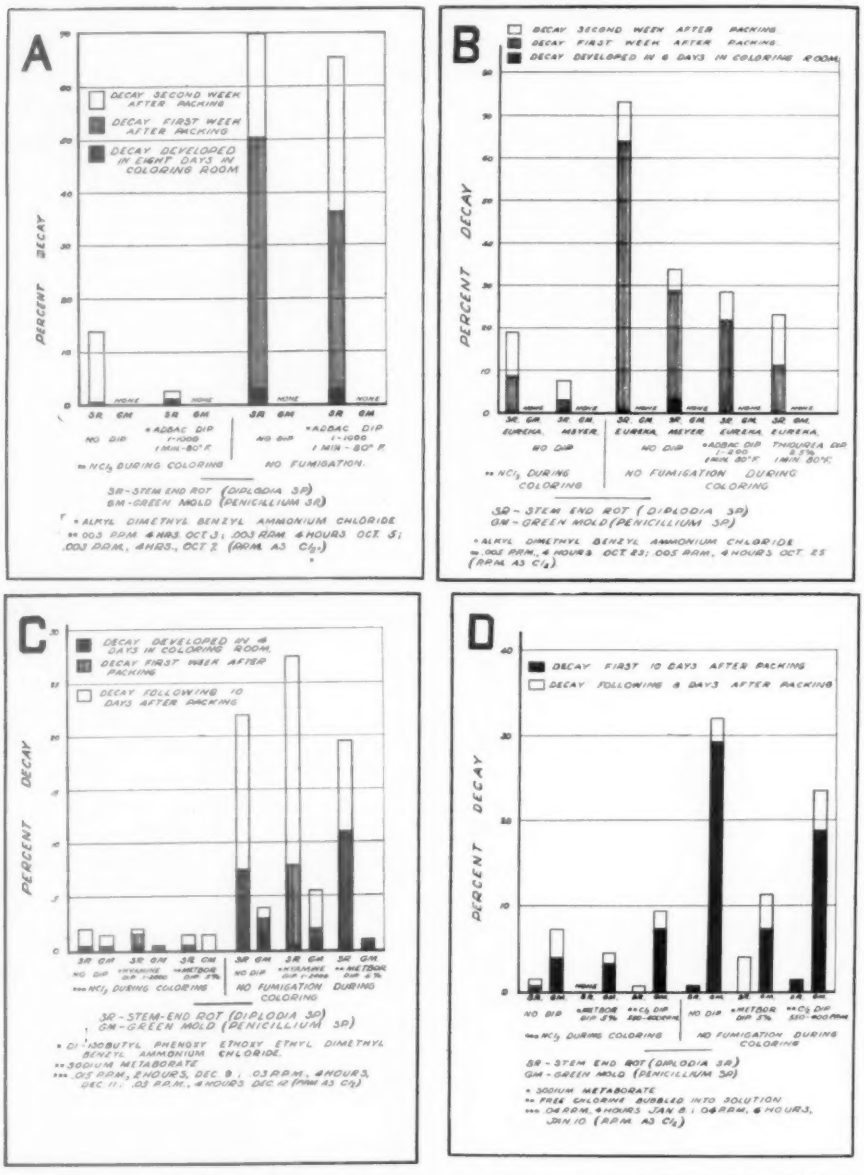
A
DECAY SECOND WEEK AFTER PACKING
DECAY FIRST WEEK AFTER PACKING
DECAY DEVELOPED IN EIGHT DAYS IN COLORING ROOM
PERCENT DECAY
NONE
NO DIP
* ADBAC DIP 1-1000 1 MIN.-80° F.
** NCl3 DURING COLORING
NO FUMIGATION
3R-STEM END ROT (DIPLODIA SP)
GM-GREEN MOLD (PENICILLIUM SP)
* ALKYL DIMETHYL BENZYL AMMONIUM CHLORIDE
** 003 P.P.M. 4 HRS. OCT 3; .003 P.P.M. 6 HOURS OCT. 31; .003 P.P.M., 4 HRS., OCT 7. (P.P.M. AS Cl2)
B
DECAY SECOND WEEK AFTER PACKING
DECAY FIRST WEEK AFTER PACKING
DECAY DEVELOPED IN 6 DAYS IN COLORING ROOM
PERCENT DECAY
EUREKA
MEYER
THIOUREA DIP 2.5% 1 MIN. 80°F.
** NCl3 DURING COLORING
NO FUMIGATION DURING COLORING
3R-STEM END ROT (DIPLODIA SP)
GM-GREEN MOLD (PENICILLIUM SP)
* ALKYL DIMETHYL BENZYL AMMONIUM CHLORIDE
** .005 P.P.M., 6 HOURS OCT 23; .005 P.P.M., 4 HOURS OCT. 25 (P.P.M. AS Cl2)
C
DECAY DEVELOPED IN 6 DAYS IN COLORING ROOM
DECAY FIRST WEEK AFTER PACKING
DECAY FOLLOWING 10 DAYS AFTER PACKING
PERCENT DECAY
* METABOR DIP 3%
** NCl3 DURING COLORING
NO FUMIGATION DURING COLORING
3R-STEM END ROT (DIPLODIA SP)
GM-GREEN MOLD (PENICILLIUM SP)
* DI-ISOBUTYL PHENOXY ETHOXY ETHYL DIMETHYL BENZYL AMMONIUM CHLORIDE
** SODIUM METABORATE
*** .015 P.P.M., 2 HOURS, DEC. 9; .03 P.P.M., 4 HOURS, DEC. 11; .03 P.P.M., 4 HOURS, DEC. 17 (P.P.M. AS Cl2)
D
DECAY FIRST 10 DAYS AFTER PACKING
DECAY FOLLOWING 6 DAYS AFTER PACKING
PERCENT DECAY
NO DIP
* METABOR DIP 3%
** NCl3 DURING COLORING
NO FUMIGATION DURING COLORING
3R-STEM END ROT (DIPLODIA SP)
GM-GREEN MOLD (PENICILLIUM SP)
* SODIUM METABORATE
** FREE CHLORINE BUBBLED INTO SOLUTION
*** .04 P.P.M., 4 HOURS JAN 8; .04 P.P.M., 6 HOURS, JAN 10. (P.P.M. AS Cl2)

FIG. 1. The control of decay in lemons after harvest as influenced by NCl_3 gas and dip treatments: A. Lemons harvested Oct. 3, 1946; B. Harvested Oct. 23, 1946; C. Harvested Dec. 9, 1946; D. Harvested January 8, 1947.

than 1 per cent stem-end rot 10 days after packing. Comparable lots treated with NCl_3 at 0.04 p.p.m. during degreening developed 4 per cent

green mold rot and about 0.5 per cent stem-end rot. Neither sodium metaborate nor chlorine-solution dip was satisfactory for control of green mold rot when such dips were the only treatments used. Results were better when both dips were followed by NCl_3; but the chlorine solution dip followed by NCl_3 had no advantage over the gas treatment alone.

DISCUSSION

Treatment of lemons with nitrogen trichloride at concentrations of 0.003 to 0.04 p.p.m. for several periods of approximately 4 hours each during the degreening process was responsible for a significant reduction in the amount of stem-end rot and blue and green mold rot which developed during the holding period after packing.

No evidence of injury to lemons was found as a result of treatment with NCl_3 at the concentrations used, and the interruptions in the ethylene gas treatment for the purpose of treating with NCl_3 appeared to have no significant effect upon the rate of degreening.

The quaternary ammonium compounds tested were of little promise when used alone; but when used as post-harvest dips followed by nitrogen trichloride treatment, both compounds gave results somewhat better than were obtained with the gas treatment alone. Sodium metaborate solution alone was partially effective for the control of both stem-end and green mold rots. However, when sodium metaborate was used in combination with NCl_3 gas the control of rot was better than with the dip alone or the gas alone. None of the solutions used as dips had any visibly adverse effects upon the lemons either when used alone or when used in combination with nitrogen trichloride. A slight bleaching resulted from the use of a dilute chlorine solution, but this added to rather than detracted from the appearance of the lemons.

There were seasonal differences in the type of decay: stem-end decay was completely dominant in October, some Penicillium decay appeared in December, and in January the green mold was predominant and there was little stem-end decay. This changing relationship undoubtedly is closely related to temperatures in the grove and in the warehouse after packing. Diplodia stem-end decay requires relatively high temperatures for optimum development, whereas green mold and blue mold fungi thrive at considerably lower temperatures. Outdoor temperatures in October averaged about 78° F., whereas in early January they averaged about 60° F.

SUMMARY

Certain chemical solutions as post-harvest dips and the use of nitrogen trichloride gas in the degreening room are discussed in connection with the handling of Eureka and Meyer lemons.

Nitrogen trichloride, in concentrations ranging from 0.003 to 0.04 p.p.m., when applied for 2 to 4 periods of approximately 4 hours each during degreening reduced stem-end and green mold rots materially.

Two quaternary ammonium compounds, sodium metaborate, and chlorine solutions were of only fair fungicidal value when used as dips at the concentrations tested; but when combined with nitrogen trichloride gas treatments in the coloring room they controlled both stem-end and green mold rots.

The relative importance of stem-end rot and green mold rot changes during the season, stem-end rot being dominant in October and green mold rot of major importance in January.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES,
U. S. DEPARTMENT OF AGRICULTURE
AND
DEPARTMENT OF PLANT PATHOLOGY,
TEXAS AGRICULTURAL EXPERIMENT STATION.

A COMPARISON OF FUNGICIDAL TREATMENTS FOR THE CONTROL OF DECAY IN CALIFORNIA CANTALOUPES

W. R. Barger, James S. Wiant, W. T. Pentzer, A. Lloyd Ryall, and D. H. Dewey[1]

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Decays of western-grown cantaloupes are not infrequently observed in shipments arriving on eastern terminal markets or during the several-day marketing period following unloading. When refrigeration is inadequate, the transit period abnormally long, or the cantaloupes unusually ripe, decay is more prevalent. The decays most commonly observed are rhizopus rot, cladosporium rot, alternaria rot, and fusarium rot.[2]

Tests were begun in 1943 and continued at irregular intervals through 1946, to determine whether some of the fungicidal treatments used with other fruits and vegetables might have value in controlling cantaloupe decays.

The studies included: (1) storage tests at Fresno, California, (2) shipping tests from Fresno (or the vicinity thereof) to New York City, and (3) laboratory tests with nitrogen trichloride at New York City.

MATERIALS AND METHODS

The following treatments were tried:

Gas treatments. Ozone, carbon dioxide, sulfur dioxide, nitrogen trichloride.

Washes. Sodium tetraborate, sodium metaborate, boric acid, sodium hypochlorite, calcium hypochlorite, merthiolate, quaternary ammonium compounds.

Dusts. Sulfur, sodium bisulphite.

Waxes. Carnauba-paraffin wax emulsion, wax-sodium bisulphite mixture.

Wraps. Diphenyl, copper-oxyquinoline.

Treated ice. Ice containing benzoic acid or chloramine.

The materials were tested by treating fairly large samples of melons, relying upon natural infection and repeated tests to establish their effectiveness. After treatment the melons in the storage tests were held at temperatures of 50° F. or above to promote the growth of the fungi causing decay. In all tests, fully mature "choice" or "hard-ripe" melons of the Powdery Mildew Resistant No. 45 variety were used.

[1] The authors are, respectively, associate physiologist, senior pathologist, senior physiologist, horticulturist, and assistant physiologist in the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

[2] Wiant, James S. Investigations of the market diseases of cantaloupes and honey dew and honey ball melons. U. S. Dept. Agr. Tech. Bul. 573. 1937.

STORAGE TESTS

In a preliminary test in 1943, cantaloupes were exposed to ozone in concentrations of 20 p.p.m. up to nine hours at 70° F. The treatment was ineffective in controlling subsequent decay in spite of the high concentration used. These results were verified in later tests (Table 1, Test 1) when ozone

TABLE 1.—*Comparison of fungicidal treatments for the control of decay in cantaloupes, 1943 season*

Treatment	No. of melons	Percentage of melons decayed
Test 1. Sept. 1–13; stored 7 days at 65°, and 5 days at 50° F.		
Untreated	45	80
CO_2 storage[a]	42	90
Ozone fumigation, 20 p.p.m., 8 hr.	56	79
Sulfur dust	45	62
Sodium bisulphite dust	45	36
Test 2. Sept. 14–30; stored 14 days, slow cooling 70° to 55° F.		
Untreated	45	91
CO_2 storage[b]	13	77
SO_2 fumigation, 0.25 per cent, 20 min.	45	76
Sodium bisulphite dust	26	73
SO_2 fumigation, 1.0 per cent, 20 min.	44	68
Wax coating (Brogdex B-123)	27	52
Sodium hypochlorite wash, 0.2 per cent Cl_2, 2 min.	14	21
Wax-bisulphite coating[c]	26	11
Borax wash, 2½ per cent, 100° F., 2 min.	12	0
Test 3. Oct. 1–18; stored 14 days (1 day 70°, 3 days 65°, 10 days 50° F.)		
Untreated	30	80
Sodium hypochlorite wash, 0.2 per cent Cl_2, ½ min.	30	44
Borax wash, 2½ per cent, 75°, ½ min., rinsed	30	40
Borax wash, 2½ per cent, 110°, ½ min., rinsed	30	20
Borax wash, 2½ per cent, 75°, ½ min., not rinsed	30	7
Borax wash, 2½ per cent, 110°, ½ min., not rinsed	30	0

[a] Refrigerator car concentrations: 17–30 per cent first 17 hours, 6–8 per cent following four days.

[b] Refrigerator car concentrations: 20–28 per cent first 17 hours, 6 per cent five days.

[c] Wax and sodium bisulphite in proportion of 75 gm.: 35 gm.

was compared with sulfur dust, sodium bisulphite dust, and carbon dioxide atmospheres comparable to those obtained in refrigerator cars in cherry shipments.[3] Sodium bisulphite dust was the best of these treatments in 1943, but other materials were superior to it in test 2 (Table 1) when it was compared with sulfur dioxide fumigation, borax and hypochlorite washes, carbon dioxide atmospheres, and waxes. In test 2, borax gave perfect control of decay for the 14-day holding period. The wax-bisulphite mixture was next in effectiveness, followed by sodium hypochlorite. The other treatments were unpromising in comparison with these three. Fumigation with

[3] Gerhardt, Fisk. A study of CO_2 gas retention in refrigerator cars during the commercial freight shipment of sweet cherries. H. T. & S. Office Rept. 75, U. S. Dept. Agr., Bur. Plant Ind., Soils, and Agr. Engin. 1941.

sulfur dioxide caused no injury of any consequence even in one per cent concentration, but it was of little value in controlling decay. The wax-bisulphite treatment, omitted in later tests, resulted in an unsightly deposit of wax on the melons owing to break down of the emulsion; there was also some browning of the stem scars. Additional tests with borax (Test 3, Table 1) demonstrated that warming the solution enhanced its fungicidal action, especially when it was not rinsed from the melons. Similar conclusions were drawn from work done on the control of citrus rots with borax washes.[4]

TABLE 2.—*Comparison of fungicidal treatments for the control of decay in cantaloupes, 1944 and 1946*

Treatment	No. of melons	Percentage of melons decayed
Test 4. Aug. 8–17, 1944; stored 6 days at 65° F.		
Untreated		
Wrapped, untreated paper	60	73
Wraps, containing 1.5 per cent Cu., 1.2 per cent oxyquinoline, 11 per cent mineral oil	60	87
Diphenyl wraps	60	88
NCl_3 fumigation, 5 hr., 11 mg. per cu. ft.	60	73
Sodium hypochlorite wash, 1.0 per cent Cl_2, 2 min.	72	32
Merthiolate wash, 1: 20,000, 2 min.	60	8
Borax wash, 5 per cent, 100° F., 2 min.	60	2
	60	0
Test 5. Aug. 17–26, 1944; stored 6 days at 65° F.		
Untreated	60	58
Sodium bisulphite wash, 2.0 per cent SO_2, 2 min.	60	42
NCl_3 fumigation, 4 hr., 15 mg. per cu. ft.	60	32
Sodium hypochlorite wash, 0.75 per cent Cl_2, 2 min.	60	20
Merthiolate wash, 1: 20,000, 2 min.	60	20
Wash, borax 2½ per cent, boric acid 2½ per cent, 75° F., 2 min.	60	8
Borax wash, 5 per cent, 100° F., 2 min.	60	2
Test 6. Sept. 17–27, 1946; stored 10 days at 50° F.		
Untreated	60	42
No-bac ice covering[a]	60	33
Cetyl trimethyl ammonium bromide wash, 1: 1,000, 2 min.	60	18
Phenolated quaternary wash, 1: 1,000, 2 min.	60	15
Lauryl dimethyl benzyl ammonium chloride wash, 1: 1,000, 2 min.	60	7
Calcium hypochlorite wash, ¼ per cent Cl_2, 2 min.	60	7

[a] Contained benzoic acid and chloramine in unknown quantities, melons covered with crushed ice three days.

Sodium hypochlorite was also used in test 3 but it was not so effective in controlling decay as borax solutions.

The tests were continued the following season using stronger concentrations of borax and of hypochlorite as well as other materials. A warm 5 per cent borax solution again gave perfect control of decay (Test 4, Table 2); next in order were merthiolate (1: 20,000 dilution), and sodium hypochlorite washes. Diphenyl wraps and copper-oxyquinoline wraps were of no ap-

[4] Winston, J. R. Reducing decay in citrus fruits with borax. U. S. Dept. Agr. Tech. Bul. 488. 1935.

preciable value. Nitrogen trichloride fumigation reduced subsequent decay to about half that in the untreated melons. These four effective treatments and a sulfurous acid solution made with sodium bisulphite were compared in another test. A warm solution of borax gave the best control, followed in effectiveness by a borax-boric acid mixture, merthiolate, sodium hypochlorite, and nitrogen trichloride (Test 5, Table 2). Other materials, including calcium hypochlorite, quaternary ammonium compounds, and a bactericidal ice, were tried in test 6 (Table 2). Calcium hypochlorite and one of the quarternary compounds gave good control of decay during a 10-day period. Covering the melons with bactericidal ice, as used commercially on fish, was of little value against cantaloupe rots.

SHIPPING TESTS

At the end of the second season's trials (1944) a test shipment of treated cantaloupes was sent to New York as part of a commercial carload of melons.

TABLE 3.—*Decay development in test shipments from California to New York, New York*

Treatment[a]	No. of melons	Percentage of melons decayed
Shipment 1. Aug. 25–Sept. 5, 1944; 12 days in transit, 4 days on market		
Untreated	89	66
Borax wash, 5 per cent, 100° F., 2 min.	63	25
Borax wash, nitrogen trichloride fumigation, 5 hr., 17 mg. per cu. ft.	90	24
Nitrogen trichloride fumigation, 5 hr., 17 mg. per cu. ft.	89	24
Shipment 2. Aug. 28–Sept. 9, 1945; 12 days in transit, 2 days on market		
Untreated	59	42
Sodium metaborate wash, 5 per cent, 78° F., 2 min.	61	10
Calcium hypochlorite wash, 0.3 per cent Cl_2, 78° F., 2 min.	60	9
Nitrogen trichloride fumigation, 6 hr., 25 mg. per cu. ft.	63	8
Shipment 3. Aug. 28–Sept. 9, 1945; 12 days in transit, 3 days on market		
Untreated	51	31
Calcium hypochlorite wash, 0.3 per cent Cl_2, 78° F., 2 min.	46	20
Nitrogen trichloride fumigation, 6 hr., 25 mg. per cu. ft.	52	10
Sodium metaborate wash, 5 per cent, 78° F., 2 min.	54	4

[a] Washes applied prior to packing of Shipment 1; Washes applied to packed crates of Shipment 2 and 3. Fumigation after packing and loading.

A warm borax wash for melons prior to packing was compared with nitrogen trichloride fumigation and with a combination of both treatments. All the treatments gave some control of decay and were about equal in effectiveness (Shipment 1, Table 3). The following season sodium metaborate (more soluble in cold water than borax) and calcium hypochlorite solutions used as dips for packed crates of melons were compared with nitrogen trichloride

fumigation in two shipments to New York. In one of the test cars all three treatments gave about the same control (Shipment 2, Table 3); in the other, sodium metaborate was the best of the three treatments (Shipment 3, Table 3).

TOLERANCE OF CANTALOUPE PATHOGENS TO NITROGEN TRICHLORIDE

In earlier tests on cantaloupes, nitrogen trichloride fumigation gave rather erratic results.[5] Concentrations employed were 5 to 10 mg. per cubic foot.[6] In one test when 12 mg. per cubic foot was tried, better control was obtained. In the trials reported here the concentrations were 11, 15, 17, and 25 mg. per cubic foot and rather consistent reduction in decay was observed.

Tolerance tests with NCl_3 on cultures and viable spores of cantaloupe pathogens were made using methods described by Klotz.[7] Higher concentrations were required to kill spores of the fungi on dried filter paper plates (some mycelium no doubt was included in the transfer) than cultures 1 to 4 days old on agar plates. Five hours' exposure to concentrations of 15 to 18 mg. NCl_3 per cu. ft. failed to give complete kill of spores of any of the fungi, which included *Alternaria* sp., two strains of *Rhizopus* sp., *Cladosporium* sp., *Fusarium* sp., and *Botrytis* sp. Higher concentrations of 20 to 22 mg. per cu. ft. were lethal except to one of the strains of *Rhizopus*. In another test, 21 to 22 mg. per cu. ft. failed to kill all spores of *Alternaria, Rhizopus,* or *Cladosporium* with an exposure of 5 hours, whereas agar cultures were killed in 1 to 3 hours.

These tests indicate that a complete kill of fungi causing cantaloupe decays cannot be expected by fumigation with NCl_3 and that mycelium in early-stage shallow infections might be easier to kill than spores. Concentrations of 20 to 23 mg. of NCl_3 per cu. ft. for 5 hours are preferable to lower concentrations and shorter exposures. The appearance of the melons was unaffected, except for greening of the sutures, by 5½ hours' fumigation with concentrations as high as 29 to 30 mg. NCl_3 per cu. ft.

DISCUSSION

The chief decays encountered in the present tests were rhizopus rot and cladosporium rot. The latter was the principal decay found at the lower temperatures. For many of the materials tested only negative results were obtained.

[5] Pentzer, W. T., James S. Wiant, and John H. MacGillivray. Market quality and condition of California cantaloupes as influenced by maturity, handling, and precooling. U. S. Dept. Agr. Tech. Bul. 730. 1940.

[6] Concentrations as given in Technical Bulletin 730 were changed to agree with corrected conversion curve of color disc readings of Cl_2 to mg. of NCl_3 worked out by the Wallace & Tiernan Company who cooperated in these tests. Credit is due H. K. Parker of that firm for his assistance in tests on toxicity of NCl_3 to spores and cultures of cantaloupe pathogens.

[7] Klotz, L. J. Nitrogen trichloride and other gases as fungicides. Hilgardia 10: 27–52. 1936.

The three treatments that showed most promise in the control of decay were sodium borate and sodium hypochlorite washes and nitrogen trichloride fumigation. Merthiolate was a good fungicide in dilute solution of 1 : 20,000 but it cannot be recommended; food authorities object to its mercury content. One of the quaternary ammonium compounds tried (lauryl dimethyl ammonium chloride) gave fairly good control but it is not known whether its use would be approved by food authorities. Fumigation with sulfur dioxide, as used for grapes, proved to be of less value than hypochlorite or borate washes.

Normally cantaloupes are packed "dry" without washing, therefore the use of antiseptic washes such as borax and hypochlorite solutions would necessitate the addition of equipment for this purpose.

Fumigation with nitrogen trichloride requires the use of a generator for manufacturing the gas as it is needed. It is not practicable to store the gas in cylinders. Treatment of entire carlots of cantaloupes with nitrogen trichloride is used commercially by several California shippers. The gas is introduced from generators into refrigerator cars soon after loading is completed. A treating period of about 5 hours is employed, with air circulation provided within the car by some type of fan.

U. S. DEPARTMENT OF AGRICULTURE,
FRESNO, CALIFORNIA.

PHYTOPATHOLOGICAL NOTES

Bird's Eye Spot Disease of Hevea Rubber in Nicaragua.[1]—In October, 1943, after a period of drought lasting approximately three weeks, there was a severe outbreak of a leaf disease of *Hevea brasiliensis* (H. B. K.) Muell. Arg. in nurseries in eastern Nicaragua. The causal organism produced lesions on the leaf blades varying from minute pinpoints to spots of 5 to 6 mm. in diameter and was identified as *Helminthosporium heveae* Petch.[2] In the initial stages the lesions are dark purple but as they enlarge the edges of the circular spots become lighter purple enclosing a greyish-

FIG. 1. Naturally infected leaf of seedling *Hevea brasiliensis* (H. B. K.) Muell. Arg. showing lesions of *Helminthosporium heveae* Petch. Note the excision of the marginal leaf tissues, the shot-hole effect, and the distortion of the leaflet at the right. Photographed at El Recreo, Nicaragua, by James T. Mitchell.

white central portion. In heavily infected leaves, the lesions coalesce and form irregular patterns (Fig. 1). Besides attacking the leaf blade, the fungus formed lesions in abundance on the succulent growing tips of 4- to 6-week-old rubber seedlings. Moreover, slightly elongated spots were common on the midrib and petioles of juvenile leaves. In advanced stages of the disease the young leaves tend to become distorted and concavely cupped, and the tips and margins of the leaves die, followed by excision of these

[1] A contribution from the Servicio Tecnico Agricola de Nicaragua, a technical agricultural service organization for eastern Nicaragua operated jointly by the Government of Nicaragua and by the Office of Foreign Agricultural Relations, U. S. Department of Agriculture. This study was made possible by funds provided through the United States Interdepartmental Committee on Scientific and Cultural Cooperation and funds from the Government of Nicaragua.

[2] The identity of this organism was confirmed by Mr. John A. Stevenson, Division of Mycology and Disease Survey, to whom the writer is grateful.

parts. Severe infections cause the abscission of the top "flush" of juvenile leaves. In older leaves the central dead portions of the lesions drop out, leaving a shot-hole appearance.

Under Nicaraguan conditions there were from 29 to 681 lesions per leaflet, and plants in several acres of nurseries containing over 500,000 seedlings from 1 to 14 months old were infected. In a single bed containing 242, 5-month-old plants, 100 per cent were infected. Of these, 7.4 per cent had lost all the leaves of the top "flush."

In an adjacent clonal garden several clonal varieties reputed to be high in latex yield were also susceptible to the disease. The average count of lesions on 10 terminal leaflets showed fewer spots than on the seedlings (Table 1), but this may be attributed to the spacing of the two types of plants and subsequent effects of environmental conditions. The seedlings were spaced one foot apart each way whereas the clones were planted in rows spaced six feet apart and the plants were spaced four feet apart in the rows.

TABLE 1.—*Clones of Hevea brasiliensis showing percentage of plants infected and the average number of lesions per terminal leaflet*

Clone	Plants infected (Per cent)	Ave. No. of lesions per leaflet
GA.–49	100	64
GA.–308	100	16
GA.–1264	100	19
GA.–1279	100	47
GV.–21	100	19
GV.–31	100	24
GV.–37	80	41
GV.–42	100	13
GX.–26	100	15

From table 1 it can be seen that none of the clonal material escaped infection even though the number of lesions per leaflet varied from a minimum of 13 to a maximum of 64 in clones GV.–42 and GA.–49, respectively.

In Nicaragua, where a large-scale budding program was in progress, it was noted that in severely infected seedlings which had lost the top "flush" leaves through defoliation, budding successes or "takes" were poor. In a trial where 100 plants with defoliated top "flushes," and a similar number of slightly infected plants that retained their foliage, were budded under identical atmospheric conditions the successes were four times greater in the latter than in the former. On the basis of this trial, defoliated plants were not budded thereafter since seedlings were available in abundance. However, had there been a scarcity of stock plants, control measures would have been required.

Although the importance of this leaf disease is insignificant when compared with that of the South American leaf blight (*Dothidella ulei* P. Henn.), yet it can be of importance in that budding successes may be de-

creased when defoliation occurs.—ARTHUR G. KEVORKIAN, formerly Senior Pathologist, Technical Collaboration Branch, Office of Foreign Agricultural Relations, U. S. Department of Agriculture. Atkins Garden and Research Laboratory, Harvard University.

Powdery Mildew Resistance in Cucumber.—Powdery mildew (*Erysiphe cichoracearum* D. C.) on cucumbers has been reported from widely separated localities of the world but is of relatively little economic importance in most of these areas. Field infection of sufficient severity to require some means of control has been reported from Palestine, Germany, and Australia, and control measures are sometimes necessary in California. No reference to resistance to this disease has been found, and apparently no systematic search for powdery mildew resistance has been made.

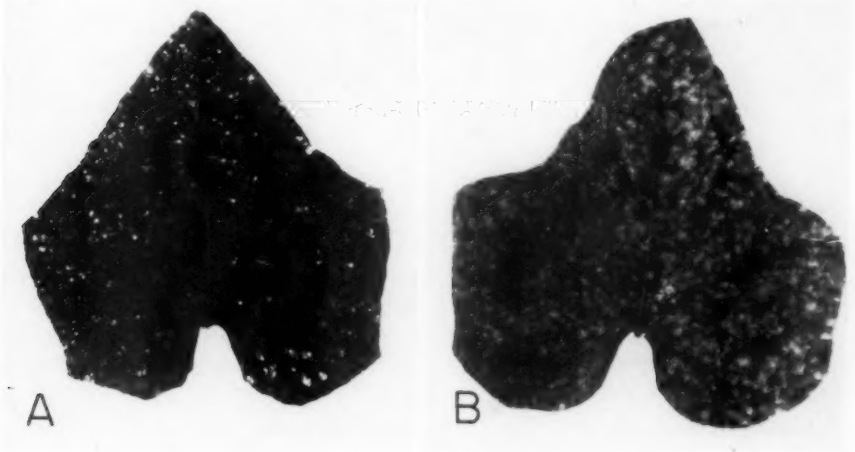

FIG. 1. Powdery mildew on cucumber. A. Leaf of Puerto Rico No. 37 with only a trace of mildew. Sharply outlined white spots are mechanical injuries. B. Leaf of susceptible Abundance variety.

In 1940 seed of Puerto Rico No. 37 was received from Dr. Arturo Roque of the Puerto Rico Agricultural Experiment Station for trial observation. Plants of this variety and of the Abundance variety were grown in the greenhouse the following winter during which powdery mildew from natural sources appeared. The Abundance variety became severely infected while the Puerto Rico No. 37 had only a trace of mildew (Fig. 1). Crosses between these two varieties were made and the F_1 grown the following summer. In the winter of 1942–43 a small population of F_1 and F_2 plants were grown in the greenhouse. These were inoculated artificially and, at about time of fruit set, rated for degree of infection, according to a system similar to that used by Pryor and Whitaker[1] in which 0 = disease free and 4 = severely diseased. No effort was made to determine which strain of mildew

[1] Pryor, D. E., and T. W. Whitaker. The reaction of cantaloupe strains to powdery mildew. Phytopath. 32: 995–1004. 1942.

was involved. In this test Snow's Perfection variety was substituted as the susceptible check instead of the Abundance variety used in the hybrid. Both varieties are highly susceptible.

Although two of the three plants of Puerto Rico No. 37 were free of disease at the time the readings were made, some disease developed on them later. The susceptible checks and hybrids by this time, however, were nearly dead from mildew. The susceptibility of the F_1 and range in susceptibility of the F_2 population show that resistance is a result of multiple factors and is not complete. In the F_2 population, 5 plants out of 110 approached the resistant parent in degree of resistance, so that, with larger F_2 populations, a degree of resistance about equivalent to that of Puerto Rico No. 37 should be obtained.

TABLE 1.—*Powdery mildew ratings of resistant, susceptible, and F_1 and F_2 hybrid progenies. Numbers are totals of plants in each class. Snow's Perfection was substituted for Abundance as the susceptible check variety. Both are highly susceptible*

Variety	Disease index				
	0	1	2	3	4
Snow's Perfection	.....	.....	.....	.....	3
Puerto Rico No. 37	2	1	.....	.....	.....
Abundance × Puerto Rico No. 37 F_1	.....	.....	.....	3	6
do F_2	.....	5	38	39	28

Inasmuch as Puerto Rico No. 37 has not been selected for resistance to powdery mildew, it might be possible within this variety to select for a higher degree of resistance than has been seen in the few plants observed. Although this variety has genes for resistance to both downy and powdery mildew, it is unlikely that downy mildew resistant varieties derived from Puerto Rico No. 37 should retain the powdery mildew resistance of that parent.—PAUL G. SMITH, University of California, Davis, California.

"Needle Curl" of Shortleaf Pine Seedlings.—A needle deformity called "needle curl" (Fig. 1) developed on about two hundred 2-year-old shortleaf pine (*Pinus echinata* Mill.) seedlings potted for a root inoculation experiment.[1] The seedlings were transplanted in April when the secondary needles on the new terminal shoots had just started to emerge from their sheaths. An anatomical study of the shoots showed that the expansion of the fascicular sheaths was inhibited by the temporary water deficiency caused by transplanting. As a result, the failure of the sheaths to open normally, caused a mechanical constriction of the growth of the needles. Subsequent growth and vigor of the seedlings was not reduced by the needle deformity. After establishment in the pot cultures, the growth of the secondary needles was normal.

[1] Jackson, L. W. R. Root defects and fungi associated with the little-leaf disease of southern pines. Phytopath. 35: 91–105. 1945.

Needle curl has been observed on an occasional shortleaf pine seedling growing on dry exposed sites in the vicinity of Athens, Georgia. During the course of recent pot-culture experiments on the cause of the little-leaf disease, it was found that needle curl can be induced on 1-year-old seedlings of shortleaf pine, and slash pine (*Pinus caribaea* Morelet) by reducing the soil moisture to the wilting point when the secondary needles are just emerging from the fascicular sheaths. Therefore, the curled growth of needles on pine seedlings is regarded as a drought symptom.

The relation of needle curl to drought stress is of particular interest because Neilson-Jones[2] attributed the formation of the concertina-like

FIG. 1. Needle curl on shoots of 2-year-old shortleaf pines.

needles associated with the fused needle disease of lodgepole pine (*Pinus contorta*) in England to a sudden reduction of the water supply at the time the needles were beginning to expand. Young[3] also reported that the concertina-like needles of pines affected with the fused needle disease in Australia were caused by a failure of the opening of the fascicles.—L. W. R. JACKSON, George Foster Peabody School of Forestry, University of Georgia.

The Occurrence of Phytophthora parasitica on Corn.[1]—Four corn plants among a group of 45 growing in the greenhouse in the winter of 1947 began

[2] Neilson-Jones, W. Fused needle disease of pines. Empire For. Jour. **20**: 151–161. 1941.

[3] Young, H. E. Fused needle disease and its relation to the nutrition of Pinus. Queensland Agr. Jour. (Australia) **53**: 45–54. 1940.

[1] Contribution from the Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Dept. of Agriculture; the Purdue University Agricultural Experiment Station; and the Missouri Agricultural Experiment Station, cooperating. Journal Paper No. 353 of the Purdue University Agricultural Experiment Station.

to show signs of wilting when 18 to 36 inches tall. The plants were growing in compost soil in 12-inch clay pots. The affected plants began to wilt during a period when the temperature and relative humidity were high. Spread of the disease to the remaining plants was stopped by lowering the temperature and humidity in the house and reducing the amount of water given the plants.

The youngest leaves in the whorl were the first to show wilting, but all the leaves on a plant were involved after 10 to 14 days. A crown rot was

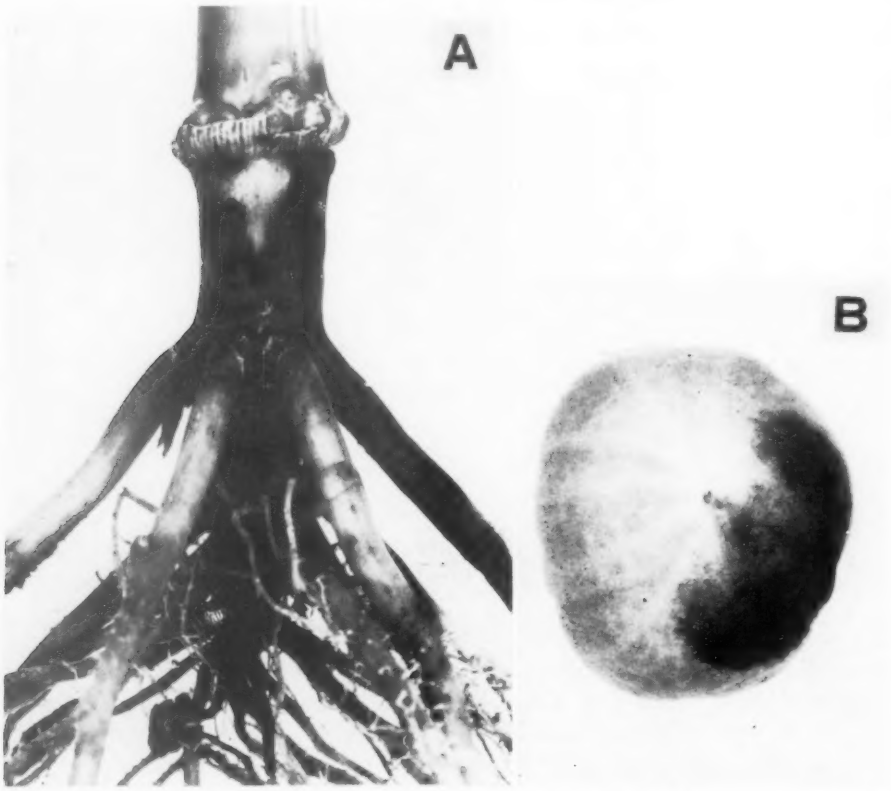

FIG. 1. A. Crown rot of a young corn plant caused by *Phytophthora parasitica*. B. Buckeye rot on green tomato fruit, 72 hours after inoculation with *P. parasitica* isolated from corn.

observed just below the soil line when affected plants were dug (Fig. 1). The rotted area was deep brown, firm, slightly depressed, and appeared to spread slowly toward both the roots and stalk.

Isolations were made on tap-water agar without difficulty. Inoculations with pure cultures of the isolate into the crowns of healthy corn plants, that ranged in height from 12 to 30 inches, resulted in typical wilting and crown rot. Mycelium of the isolate placed over corn kernels planted in sand induced complete rotting before emergence. Inoculations into healthy

Grimes Golden apples produced a soft, brown, rotted area 4 to 5 cm. in diameter after 96 hours of incubation. Only a very limited invasion of potato tubers took place in the same time. Mycelium of the isolate placed on the unwounded surface of green tomato fruits produced typical symptoms of buckeye rot within 72 hours (Fig. 1).

The fungus produced an abundance of sporangia and zoospores in sterile distilled water after previous nourishment on sterile pea broth. Zoospores, which were ejected directly from the sporangia, were oval to kidney-shaped and bore two flagella. The fungus grew well on several of the common vegetable extract agars at 24° to 26° C. On grated carrot agar, the sporangia were 47–78 $\mu \times 21$–46 μ with means of 49.2 $\mu \times 32.1$ μ. Chlamydospore diameter was 18–40 μ with a mean of 26.3 μ. No oogonia or antheridia have been observed on any substrate.

From its morphology, cultural behavior, growth at critical temperatures, and from symptoms produced on tomato fruits, the pathogen has been identified as *Phytophthora parasitica* Dastur. An isolate of the same species obtained from soil in a greenhouse at Columbia, Missouri, in which buckeye rot was prevalent in a recent tomato crop, was used for comparative inoculations. Both isolates proved pathogenic to both corn and tomatoes. In wound inoculations on both hosts, the isolate from corn was significantly more virulent than the one from tomato; the differences, however, were quantitative. There were no definite differences that might be considered of sufficient importance to distinguish the isolates as physiologic forms.

Comparative studies on the morphology of the isolates from corn and tomato revealed only minor differences in the ratio of sporangium length to diameter and in the diameter of chlamydospores. The differences fell well within the range for the species as reported by the junior author.[2]

The occurrence of the genus *Phytophthora* as a parasite on corn is unusual. Clinton[3] reported *P. cactorum* on corn, basing his identification on the presence of oospores in the rotted roots. His figures, and the subsequent studies on root rots of corn, suggest that the oospores may have been those of a species of *Pythium*. Sideris[4] found an unidentified species of *Phytophthora* to be weakly parasitic when inoculated into corn roots.—ARNOLD J. ULLSTRUP, Department of Botany and Plant Pathology, Purdue University Agricultural Experiment Station, Lafayette, Indiana, and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Dept. Agriculture; and C. M. TUCKER, Department of Botany, University of Missouri, Columbia, Mo.

A simple method of preserving tobacco-mosaic sera.—In our laboratory the immune sera of tobacco mosaic virus used to be stored in sterile tubes

[2] Tucker, C. M. The taxonomy of the genus *Phytophthora*. Mo. Agr. Exp. Sta. Res. Bul. 53. 1931.

[3] Clinton, G. P. New or unusual plant injuries and diseases found in Connecticut, 1916–1919. Conn. Agr. Exp. Sta. Bul. 222: 397–482. 1920.

[4] Sideris, C. P. Taxonomic studies in the family Pythiaceae II. Pythium. Mycol. 24: 14–61. 1932.

and preserved by refrigeration in an ice-chest, because the addition of preservative was not advisable. It was found recently, however, that the following method was also promising for preserving the sera. The immune sera, about 0.5 cc. in volume, were distributed into sterilized test-tubes, 5 cm. long and 0.8 cm. wide, and immediately dried *in vacuo* by use of sulphuric acid. The sera can be perfectly dried within several hours, or overnight at the longest, and they stick fast to the glass wall of the tube. All the tubes thus treated were then plugged, sealed with paraffin, and stored in a laboratory-chest (the cooler the better). At the time of use an exact amount of distilled water is added to make the serum concentration the same as the original, and the tube is kept at room temperatures (15° – 30° C). It takes several hours to make all the serum sticking to the glass wall dissolve in water, provided the sealed tubes have been kept in a cool place. The sera when completely dissolved are of the same transparency as when frozen, or sometimes very slightly turbid as compared with frozen sera.

TABLE 1.—*The efficacy of dried and frozen antisera for tobacco mosaic*

Antisera[a]	Method and period of preservation		Date of serological test	Precipitin reaction[b] and dilution of sera
		Days		
A	Dried	232	Apr. 13, 1948	+ at 1/320
	Frozen	do	do	+ at 1/320
B	Dried	306	June 26, 1948	± (−) at 1/320
	Frozen[c]	do	do	± at 1/40

[a] The antiserum was obtained from a rabbit that was bled Aug. 24, 1947.
[b] The + indicates a distinct precipitin reaction; ± indicates a slight precipitation; and (−) indicates a tendency toward a negative reaction.
[c] The sample thawed for some time during storage because of refrigerator trouble.

The dissolved serum is ready for serological study. The results of some experiments with dried sera in comparison with frozen sera are in table 1.

The titre of the dried antiserum A was not reduced in comparison with that of the frozen antiserum A, when tests were made after 232 days. The titre of the dried antiserum B was not much reduced after 306 days. The marked reduction in titre of the frozen antiserum B may be attributed to the accidental thawing of the serum during storage.—T. MATSUMOTO and CHIN-HOW LEE, National Taiwan University, Taipeh, Formosa, China.

A Seedling Blight of Avocado Caused by Phytophthora palmivora.—In October, 1947, the writer's attention was called to an unusual disease of avocado seedlings growing in a slathouse of a large nursery near Princeton, Florida. Nearly all of approximately 4,000 seedlings, grown for use in grafting, were severely attacked by a blight that rendered them unfit for use as rootstocks. All of the aerial parts of the plants were attacked to some degree, as well as the scions of plants already grafted. The seedlings,

grown from seed of several varieties, showed no observable differences in reaction. This outbreak occurred during a period of heavy rainfall and high humidity.

Affected matured leaves had large, irregular, reddish-brown necrotic areas that appeared to enlarge most rapidly in the direction of the major veins (Fig. 1). Lesions in young leaves were darker in color, and such leaves were usually curled or twisted. The terminal bud was frequently attacked and killed. Stem lesions, observed only on succulent stems, were dark brown, elongate, sunken, and appeared to result from the progress of

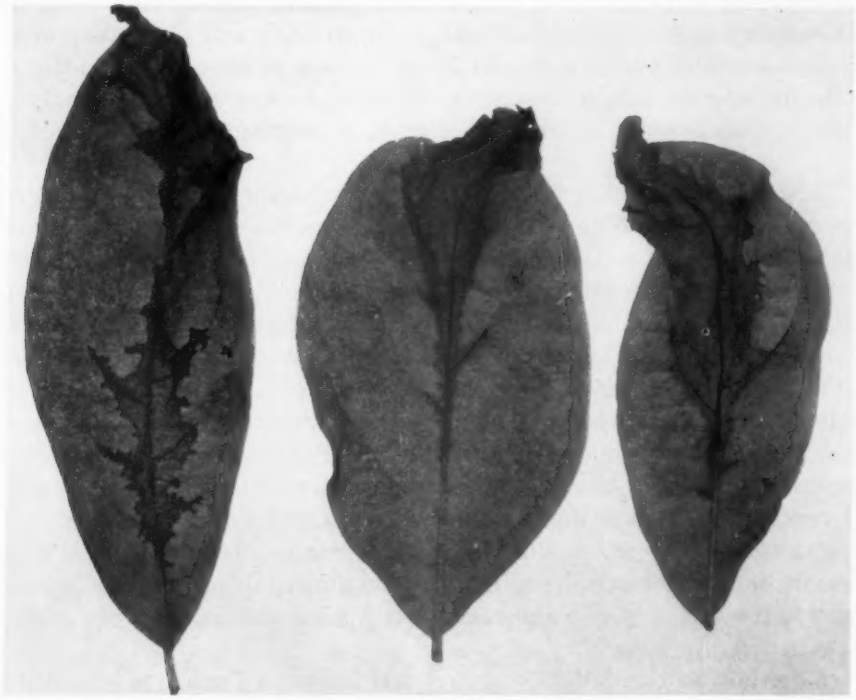

FIG. 1. Leaves of avocado seedlings with symptoms resulting from natural infection with *Phytophthora palmivora*.

the disease into the stem from the petioles. Stem lesions frequently caused the stem to break and occasionally led to the death of the seedling.

Conidia of a *Phytophthora* were constantly found in the diseased areas, though not in abundance. Isolations from the margins of diseased areas uniformly yielded pure cultures of *Phytophthora palmivora* Butler.[1] Uninjured avocado seedlings were inoculated on the leaves and stems by atomizing a conidial suspension obtained from cultures of this fungus, and the inoculated plants were covered with a bell-jar for 48 hours. Individual lesions were at first watersoaked and generally circular until a major vein was contacted. Subsequent enlargement of a lesion was most rapid along the

[1] The author is indebted to Dr. C. M. Tucker, Department of Botany, University of Missouri, for the identification of this fungus.

veins. Stem infections resulted from the progress of the fungus into the stem from the leaf. Symptoms, as seen in the nursery, were evident after 5 to 7 days. The fungus was readily recovered from the lesions.

An examination of the available literature disclosed only one other record of the avocado being a host of this fungus. Tucker[2] listed avocado as a host of *P. palmivora* in the Philippines and cited Reinking[3] as the authority. An examination of Reinking's publication revealed, however, that he did not designate the species of *Phytophthora* involved, but merely stated that: "It resembles somewhat the fungus that produces coconut bud rot, black rot of cacao, and the rots of various other plants." While Reinking did not specifically identify the pathogen, his description and illustration of the avocado seedling blight in the Philippines make it appear to be identical with the disease herein described.—ROBERT A. CONOVER, University of Florida, Sub-Tropical Experiment Station, Homestead, Florida.

Sample Size and Plot Size For Testing Resistance of Strawberry Varieties to Verticillium Wilt[1].—During recent years the development of varieties having resistance to verticillium wilt has been an important objective of the strawberry breeding program of the University of California College of Agriculture. At the outset much time was devoted to a study of this disease in California[2] and to the development of a "wilt nursery." Parental material was classified in a general way according to its resistance. In 1945 a study of the results of previous years indicated considerable variation among plants in the same plot. The system of classification used apparently had been satisfactory for types that were either very susceptible or very resistant. For the intermediate types with greater variation, however, a more accurate measure of resistance was necessary. Because it was thought that larger samples might give the desired information, this paper reports the accuracy of measurement with plots and samples of different sizes.

Selections and varieties (of × *Fragaria ananassa* Duch.) to be tested in the field were planted in soil infested with inoculum from different localities in the State. In addition each plant was inoculated with the causal organism (*Verticillium alboatrum* R. and B.) at transplanting time. The roots of each plant were thus brought into close contact with fresh inoculum and inoculated soil. By this procedure 3885 plants were treated in 1945, 1975 in 1946, and 5564 in 1947. Only results for the commercial varieties in 1946 and 1947 are reported.

Planting was delayed until late April each year to avoid, as much as

[2] Tucker, C. M. The distribution of the genus *Phytophthora*. Mo. Agr. Exp. Sta. Res. Bul. 184. 1933.

[3] Reinking, O. A. Citrus diseases in the Philippines, Southern China, Indo-China and Siam. Phil. Agr. 9: 121–179. 1921.

[1] The authors gratefully acknowledge the suggestions made by Professors G. A. Baker, L. D. Leach, and B. A. Rudolph, and the assistance rendered by Alfred Amstutz.

[2] Thomas, Harold E. Verticillium wilt of strawberries. Calif. Agr. Exp. Sta. Bul. 530. 1932.

possible, infection with "strawberry yellows." Experience indicates that plants set after mid-April are usually unaffected by "yellows" the first year and remain healthy longer than plants set early in the season.[3]

During late September of each year all plants were classified into two groups according to degree of resistance. Plants in group I had poor or only fair resistance and were not expected to live through the second season. Plants in group II had good to excellent resistance and were expected to survive and to produce fair to good crops the subsequent season.

The varieties studied had a broad range of resistance. The ratio of group I to group II plants for each variety is as follows: Lassen[4] 1946 = 3.082 : 1, Shasta 1946 = 1.299 : 1, Sierra 1947 = 0.0204 : 1. This range of resistance is characteristic of commercial varieties which have been tested.

Chi-square values were calculated for the Lassen and Shasta varieties for 1946. Total sample sizes of 75, 100, 125, 150, 175, and 200 were used.

TABLE 1.—*Heterogeneity of wilt reaction in Lassen and Shasta varieties of strawberry as determined in 1946 with constant total sample size (150 plants) and with variable plot size*

Number of plots	Number plants per plot	Number random samples (out of 20) for which P=5 per cent or less	
		Lassen	Shasta
30	5	1.5	5.0
15	10	2.0	8.0
6	25	1.5	2.5
3	50	0.0	0.0

The effect of plot size was calculated, using 5, 10, 25, and 50 plants for each unit. Forty random samples were taken for each specific combination of plot size and sample size.

The Sierra variety is so highly resistant that only 16 plants out of 800 could be classified in group I. The expected number of plants in group I would therefore be too small for the sample sizes used for other varieties. The Sierra variety represents essentially a homogeneous population of resistant plants. The entire planting of each variety was considered to be the "true" or "parent" population from which samples were drawn. Chi-squares were measures of the divergence of the sample values from the corresponding values of the population composed of all plants. The P values for the chi-squares were calculated. Only those random samples which had P values of 5 per cent or less are in tables 1 and 2.

The sample sizes of 25 and 50 plants show the least amount of heterogeneity for the varieties (Table 1). Apparently the total sample size of 150 plants for plots of 50 is sufficient to reduce the heterogeneity to a sat-

[3] Thomas, Harold E. The production of strawberries in California. Calif. Agr. Ext. Serv. Cir. 113. 1939.

[4] ———— and Earl V. Goldsmith. The Shasta, Sierra, Lassen, Tahoe, and Donner strawberries. Calif. Agr. Exp. Sta. Bul. 690. 1945.

isfactory level (1 out of 20). The sample size for plots of 25 should be studied to determine the number of plots required to increase the homogeneity so that 95 per cent or more of the random samples will have a P value greater than 5 per cent. In table 2, the effect of smaller and larger total sample sizes on the plot size of 25 plants is shown.

For Lassen 1946 and Shasta 1946 the total sample sizes of 75, 100, and 125 plants indicate a similar degree of heterogeneity, which is somewhat higher than that for larger samples. For both varieties the number of random samples indicating significant heterogeneity progressively decreases through the total sample sizes of 150, 175, and 200. In the latter, 19 out of 20 of the random samples for both varieties have P values above the 5 per cent

TABLE 2.—*Heterogeneity of wilt reaction in Lassen and Shasta varieties of strawberry as determined in 1946 with variable total sample size and constant plot size (25 plants)*

Number plots each with 25 plants	Number random samples (out of 20) for which P = 5 per cent or less	
	Lassen	Shasta
3	2.5	3.0
4	3.0	2.5
5	3.5	2.0
6	1.5	2.5
7	1.5	1.5
8	1.0	1.0

point. The homogeneity for 8 plots of 25 plants therefore falls within the commonly accepted statistical limits.

The plot sizes of 25 or 50 plants indicated a high degree of homogeneity for the variety-plot combinations. Apparently it is necessary to use a total sample of 150 plants for plots of 50, or a total sample of 200 plants for plots of 25 in order to reduce the discrepancy between the observed and the expected sufficiently to indicate homogeneity (19 out of 20).

By designing our field trials in the "wilt nursery" to include a minimum of 3 plots of 50 plants or 8 plots of 25 plants for all selections and varieties of strawberry it will be possible to determine relatively small differences in resistance among selections and varieties. Such determinations are necessary to evaluate the parental breeding material used for the development of wilt resistant varieties. They are also necessary for a study of the inheritance of wilt resistance.

Only the variety Sierra has sufficient resistance to be used for profitable commercial plantings in soil infested with verticillium wilt. Commercial plantings of the Lassen and Shasta varieties have not been profitable in soil so infested. The results indicated in our "wilt nursery" have therefore been verified by commercial results.—RICHARD E. BAKER and VICTOR VOTH, Division of Pomology, California Agricultural Experiment Station, Davis, California.

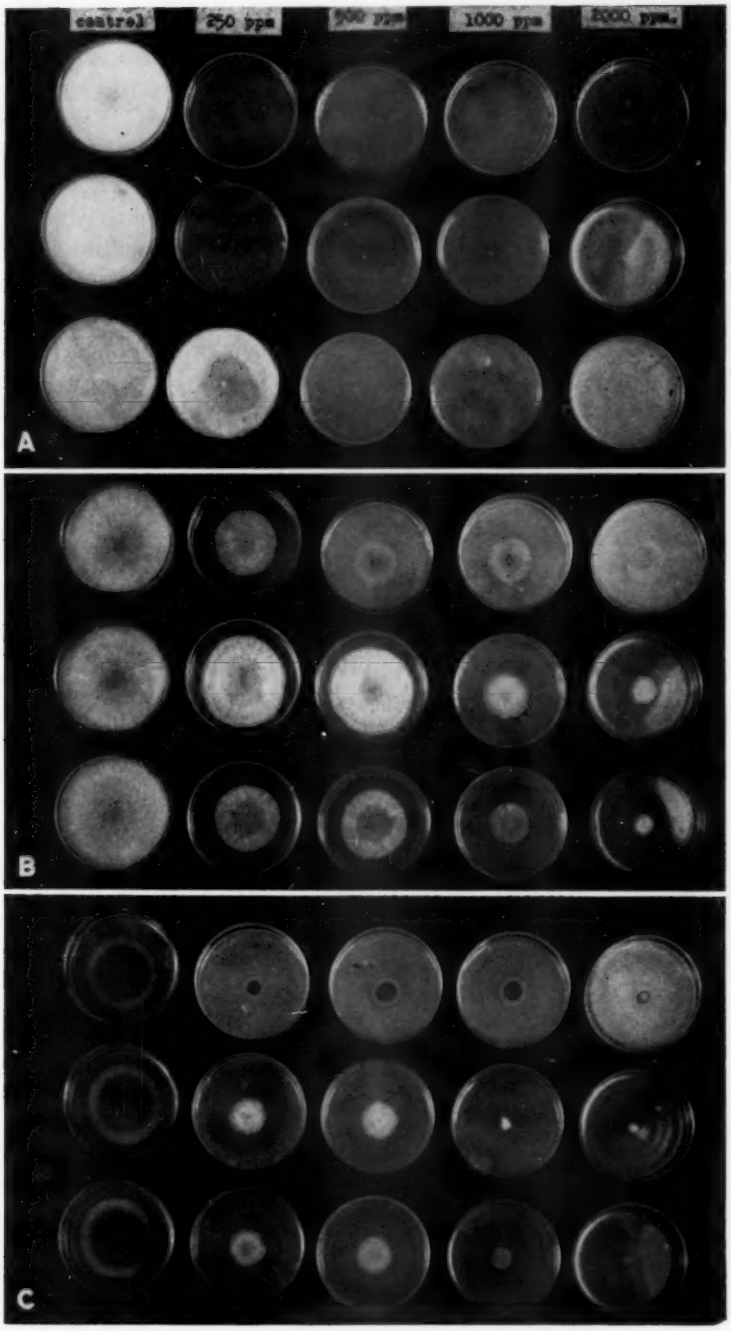

FIG. 1. Effect of 2,4-D on growth of three fungi. A, *Pythium debaryanum* top row, sodium salt, middle row, amine, bottom row, butyl ester; B, *Gibberella zeae*, top row, butyl ester, middle row, amine, bottom row, sodium salt; C, *Helminthosporium victoriae*, arrangement same as for B. All cultures six days old.

Effect of 2,4-D in Culture Medium on the Growth of Three Pathogenic Fungi.—Increasing knowledge on the effect of the 2,4-D formulations on inbred lines of corn and the small grains stimulated interest in a preliminary study on the effect of 2,4-D on fungus growth in culture. This report describes the result secured on the effect of 4 dilutions with 3 formulations of 2,4-D on 3 common genera of pathogenic fungi.

The sodium salt (Sodium Dichlorophenoxyacetate monohydrate), butyl ester (Butyl-Ester 2,4-Dichlorophenoxyacetate), and the amine (Triethanolamine-2,4-Dichlorophenoxyacetate) were each prepared in dilutions of 250, 500, 1000, and 2000 parts per million of the active acid. Ordinary potato-dextrose agar was liquefied by heating in an autoclave at 240° F., then cooled to 77° F. before the 2,4-D was added. The agar and 2,4-D formulations were thoroughly mixed and 20-cc. aliquots were poured into 15 different Petri-dishes for each formulation and dilution. When the agar solidified, 5 dishes of each formulation and dilution were inoculated with cultures of *Gibberella zeae*, 5 with *Helminthosporium victoriae*, and 5 with *Pythium debaryanum*. Three different tests were made, each with 5 replications. Controls containing no 2,4-D were run with each test.

The results can best be seen in figure 1, A, B, and C. All dilutions of the sodium salt and the amine killed *Pythium debaryanum*, while the butyl ester retarded growth in proportion to the dilution of 2,4-D. *Gibberella zeae* and *Helminthosporium victoriae* were not killed by any of the dilutions employed in this experiment, but the growth of both was severely retarded in proportion to the dilution of 2,4-D.—WAYNE M. BEVER, Cereal Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and F. W. SLIFE, Assistant, Department of Agronomy, University of Illinois, Urbana, Illinois.

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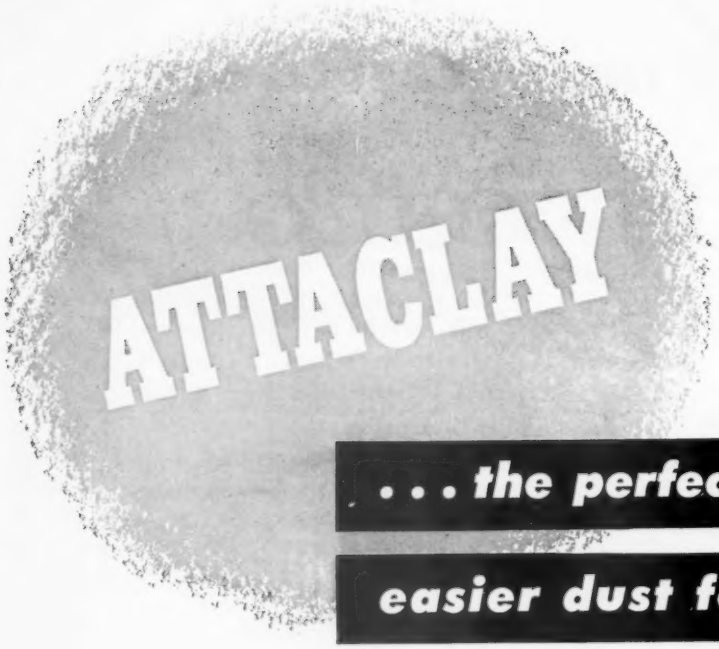

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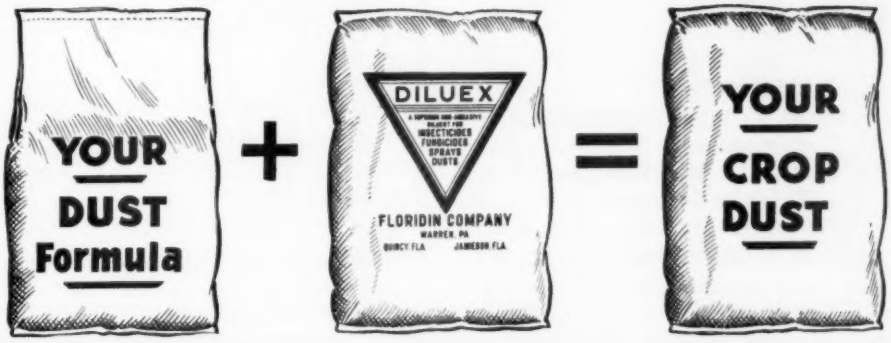

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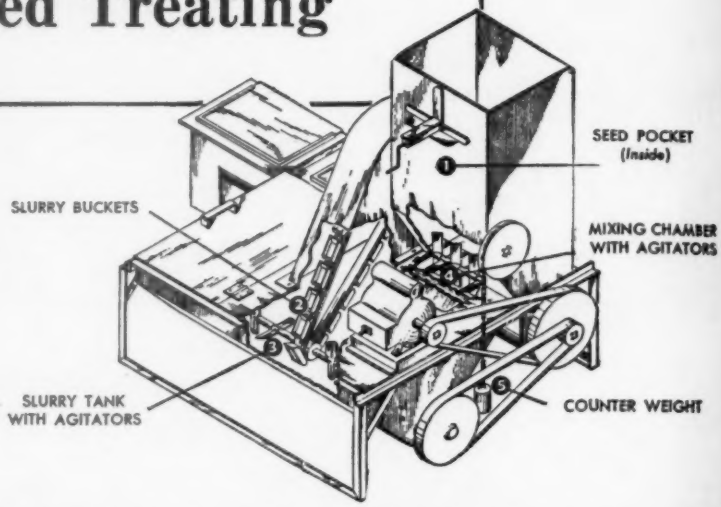

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Baker, Kenneth F., California Agricultural Experiment Station, 405 Hilgard Avenue, Los Angeles 24, California.

Baker, Richard E., Division of Pomology, University of California, Davis, California.

Bald, J. G., 107 Hilgard Hall, University of California, Berkeley 4, California.

Baldwin, I. L., College of Agriculture, University of Wisconsin, Madison 6, Wisconsin.

*__Ballard, W. S.__, 137 Cornell Street, Fresno 4, California.

Bamberg, R. H., Department of Agronomy, Montana State College, Bozeman, Montana.

Banfield, W. M., Department of Botany, Massachusetts State College, Amherst, Massachusetts.

Bardin, Roy, 71 Blanco Road, Salinas, California.

Barker, H. D., Plant Industry Station, Beltsville, Maryland.

Barnes, W. C., Box 337, Charleston, South Carolina.

Barnett, H. L., Department of Plant Pathology, West Virginia University, Morgantown, West Virginia.

Barratt, Raymond W., Department of Biology, Stanford University, Stanford, California.

*__Barre, H. W.__, Bureau of Plant Industry, U. S. Department of Agriculture, Washington 25, D. C.

*__Barrett, James T.__, Division of Plant Pathology, University of California, Berkeley 4, California.

*__Barrus, M. F.__, Department of Plant Pathology, Cornell University, Ithaca, New York.

*__Barss, H. P.__, Office of Experiment Stations, U. S. Department of Agriculture, Washington 25, D. C.

Bart, George J., Botany and Zoology Building, Ohio State University, Columbus 10, Ohio.

Baskin, A. David, Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

Batista, A. Chaves, Instituto de Pesquisas Agronômicas Dois Irmãos, Recife, Pernambuco, Brazil.

Bauer, Allen H., Botany Department, Pennsylvania State College, State College, Pennsylvania.

Baur, Karl, Western Washington Experiment Station, Puyallup, Washington.

Baxter, Dow Vawter, School of Forestry and Conservation, University of Michigan, Ann Arbor, Michigan.

*__Beach, W. S.__, Botany Department, Pennsylvania State College, State College, Pennsylvania.

Beale, Helen Purdy, Boyce Thompson Institute, Yonkers 3, New York.

*__Beattie, R. Kent__, Piney Branch Farm, North Glen Road, Route 1, Rockville, Maryland.

Beckman, Carl H., Plant Pathology Department, University of Wisconsin, Madison 6, Wisconsin.

Beecher, F. S., Division of Fruit and Vegetable Crops and Diseases, Plant Industry Station, Beltsville, Maryland.

Bell, Frank H., Department of Botany, Ohio State University, Columbus 10, Ohio.

Bell, Milton R., Cowell, California.

Bennett, Carlyle W., U. S. Department of Agriculture, Sugar Plant Laboratory, Riverside, California.

Bergman, Herbert F., East Wareham, Massachusetts.

Berk, Sigmund, 3403 Frankford Avenue, Philadelphia 34, Pennsylvania.

Berkeley, G. H., Officer in Charge, Dominion Laboratory of Plant Pathology, Box 87, St. Catherines, Ontario, Canada.

Bessey, E. A., Botany Department, Michigan State College, East Lansing, Michigan.

Bever, Wayne M., Department of Agronomy, Illinois Agricultural Experiment Station, Urbana, Illinois.

Bhide, Vishnu P., College of Agriculture, Poona 5, India.

Bickerton, Jack M., c/o Walker Fertilizer Co., Orlando, Florida.

Bingham, Richard T., 618 Realty Building, Spokane 8, Washington.

*__Bisby, G. R.__, Imperial Mycological Institute, Ferry Lane, Kew, Surrey, England.

Bitancourt, A., Caixa Postal 119–A, São Paulo, Estado de São Paulo, Brazil.

*__Black, Lindsay M.__, Brooklyn Botanic Garden, 1000 Washington Avenue, Brooklyn 25, New York.

*__Blair, R. J.__, Springdale Farm, Ormstown, Que., Canada.

Blank, Lester M., Division of Plant Pathology, Texas Agricultural Experiment Station, College Station, Texas.

Blasberg, Charles H., University of Vermont, Burlington, Vermont.

Bliss, Donald E., Citrus Experiment Station, Riverside, California.

Blodgett, Earle C., Prosser Branch Station, Prosser, Washington.

*Blodgett, F. M., Department of Plant Pathology, Cornell University, Ithaca, New York.

Bock, J. F., Federal Building, Oregon City, Oregon.

Bockstahler, H. W., Box 226, East Lansing, Michigan.

Bodine, E. W., 100 Bush Street, c/o H O Technical Products, San Francisco 6, California.

Boeshore, Irwin P., 708 Shadeland Avenue, Drexel Hill, Pennsylvania.

Boewe, G. H., 337 Natural Resources Building, Urbana, Illinois.

Bohn, Weston, Box 150, La Jolla, California.

Bollenbacher, Katharina, 2150 Pennsylvania Avenue, N. W., Washington 7, D. C.

Bonar, Lee, Department of Botany, University of California, Berkeley 4, California.

Bonde, Reiner, Orono, Maine.

Boone, Donald M., Plant Pathology Department, University of Wisconsin, Madison 6, Wisconsin.

Boosalis, Michael G., Division of Plant Pathology, University Farm, St. Paul 1, Minne ota.

Boothroyd, Carl W., Department of Plant Pathology, Cornell University, Ithaca, New York.

Borders, Huey I., c/o U. S. Department of Agriculture Laboratory, Georgia Coastal Plain Experiment Station, Tifton, Georgia.

Borlaug, Norman E., Rockefeller Foundation, Calle Viena 26, Mexico, D.F., Mexico.

*Bourne, B. A., Box 6368, Clewiston, Florida.

Bowman, Donald H., Division of Plant Pathology and Physiology, Texas Agricultural Experiment Station, College Station, Texas.

Boyce, John S., Osborn Botanical Laboratory, Yale University, New Haven, Connecticut.

*Boyd, O. C., Clark Hall, Massachusetts State College, Amherst, Massachusetts.

Boyle, Alice McL., Department of Plant Pathology, University of Arizona, Tucson, Arizona.

Boyle, John S., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

*Boyle, Lytton W., Department of Botany, Georgia Agricultural Experiment Station, Experiment, Georgia.

Bozoglu, Mrs. Saadet, Merkez Zirest Mucadela Enstitusu, Ankara, Turkey.

Bragonier, W. H., Department of Botany and Plant Pathology, Iowa State College, Ames, Iowa.

Brandes, Gordon A., c/o Rohm and Haas Company, West Washington Square, Philadelphia 5, Pennsylvania.

Brandwein, Paul F., 68–38 Yellowstone Boulevard, Forest Hills, New York.

*Brann, J. W., Baileys Harbor, Wisconsin.

*Bratley, Cyril O., Room 1022, 641 Washington Street, New York 14, New York.

Braun, Alvin J., New York State Agricultural Experiment Station, Drawer 353, Geneva, New York.

Braun, Armin C., Ridgeview Road, Princeton, New Jersey.

Bremer, Hans, Kecioren Coraklik Baglari No. 20, Ankara, Turkey.

Brentzel, W. E., State College Station, Fargo, North Dakota.

Bretz, T. W., Botany Department, University of Missouri, Columbia, Missouri.

Brian, Charles E., c/o California Spray Chemical Corp., 150 Bayway, Elizabeth, New Jersey.

Bridgmon, George H., Wyoming Agricultural Experiment Station, Powell, Wyoming.

Brierley, Philip, Plant Industry Station, Beltsville, Maryland.

Briggs, Fred N., Division of Agronomy, University Farm, Davis, California.

Brinkerhoff, Lloyd A., U. S. Cotton Field Station, Shafter, California.

Brisley, Harold R., P. O. Box 432, Douglas, Arizona.

Bridgers, Thomas F., 909 Anderson Street, Wilson, North Carolina.

Broadfoot, W. C., Division of Botany and Plant Pathology, Central Experimental Farm, Ottawa, Ontario, Canada.

*Brooks, A. N., P. O. Box 522, Lakeland, Florida.

*Brooks, Charles, Brogdex Company, 1441 West Second Street, Pomona, California.

Brown, Clarke G., Box 941, Caldwell, Idaho.

*Brown, J. G., Box 4672, University of Arizona, Tucson, Arizona.

*Brown, Nellie A., 920 Lee Street, Thompson, Georgia.

Brown, Mrs. Pembroke H., 1104 West Daniel Street, Champaign, Illinois.

Bruehl, George W., Department of Plant Pathology, South Dakota State College, Brookings, South Dakota.

Bruer, Howard L., 616 Mulberry Street, Macon, Georgia.

Bucha, Harry C., Shipley Road, R. D. 3, Wilmington, Delaware.

*Buchholtz, **Walter F.**, Department of Botany, Iowa State College, Ames, Iowa.

Bulger, Raymond O., 111 Federal Office Building, Minneapolis 1, Minnesota.

Burke, Leo James, c/o Bureau of Entomology and Plant Quarantine, 844 Federal Building, 641 Washington Street, New York, New York.

Burke, O. D., Botany Department, Pennsylvania State College, State College, Pennsylvania.

*Burkholder, **W. H.**, Department of Plant Pathology, Cornell University, Ithaca, New York.

*Burrell, **Arthur B.**, Department of Plant Pathology, Cornell University, Ithaca, New York.

Butler, Edward E., Botany Department, Michigan State College, East Lansing, Michigan.

Butler, Karl D., G. L. F. School of Cooperative Administration, Ithaca, New York.

*Byars, **Luther P.**, Marion, South Carolina.

Calavan, E. Clair, Citrus Experiment Station, Riverside, California.

Calderón, José Guevara, Oficina de Estudios Especiales, Dirección General de Agricultura, San Jacinto, Mexico, D. F., Mexico.

Caldis, P. D., 101 California Street, San Francisco 19, California.

Caldwell, Ralph M., Botany Department, Purdue University, Lafayette, Indiana.

Calvert, O. H., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

Camp, W. B., Jr., Route 1, Box 500, Bakersfield, California.

Campbell, Colin E., R. T. Vanderbilt Company, East Norwalk, Connecticut.

Campbell, Leo, Western Washington Experiment Station, Puyallup, Washington.

Campbell, Thomas H., Botany and Zoology Building, Ohio State University, Columbus 10, Ohio.

Campbell, W. A., School of Forestry, University of Georgia, Athens, Georgia.

Canero, Benjamin O., Oficina de Estudios Especiales, San Jacinto, D. F., Mexico.

Cannon, Orson S., H. J. Heinz Co., Bowling Green, Ohio.

Cano, Judn M., Botany Department, Louisiana State University, Baton Rouge 3, Louisiana.

Carlton, William M., Department of Botany, University of Georgia, Athens, Georgia.

Caron, Omer, Department of Agriculture, Quebec, Canada.

Caroselli, Nestor E., Bartlett Tree Research Laboratory, Stamford, Connecticut.

Carpenter, John B., Estacion Experimental Agricola de Tingo Maria, c/o American Embassy, Lima, Peru.

Carpenter, T. R., Agricultural Experiment Station Building, Purdue University, Lafayette, Indiana.

Carrera, J. M. Cesar, Morelo 11, Buenos Aires, Argentina.

Carsner, Eubanks, Box 31, Riverside, California.

Carter, Edward P., Georgia Agricultural Experiment Station, Experiment, Georgia.

Carter, J. C., 337 Natural Resources Building, Urbana, Illinois.

Carter, Walter, P. P. C. A. Experiment Station, University of Hawaii, Honolulu, Hawaii.

Carvajal, Fernando, Schenley Research Institute, Lawrenceburg, Indiana.

Cash, Edith K., Plant Industry Station, Beltsville, Maryland.

Cash, Lillian C., Plant Industry Station, Beltsville, Maryland.

Cassell, R. C., Department of Agriculture, Arkansas State College, Jonesboro, Arkansas.

Castro, Javier Dieguez, Botany Department, Louisiana State University, Baton Rouge, Louisiana.

Castronovo, Ada M., Yerbal 2621, Buenos Aires, Argentina.

Cation, Donald, Botany Department, Michigan State College, East Lansing, Michigan.

Cavanagh, A. E., 319 East Lullwood St., San Antonio, Texas.

Cenóz, Hugo P., Estacion Experimental, Central Castelav, F. C. O., Argentina.

Chamberlain, Donald W., U. S. Regional Soybean Laboratory, 205 Old Agricultural Building, Urbana, Illinois.

Chambers, Ernest L., Room 424 N.E., State Capitol, Madison 6, Wisconsin.

Chandler, Webster A., Botany Building, State College, Pennsylvania.

Chao, S. John, Box 121, Clear Lake, Iowa.

Chapman, Clarence M., 618 Realty Building, Spokane, Washington.

Chapman, Richard A., Department of Plant Pathology, Connecticut Agricultural Experiment Station, New Haven, Connecticut.

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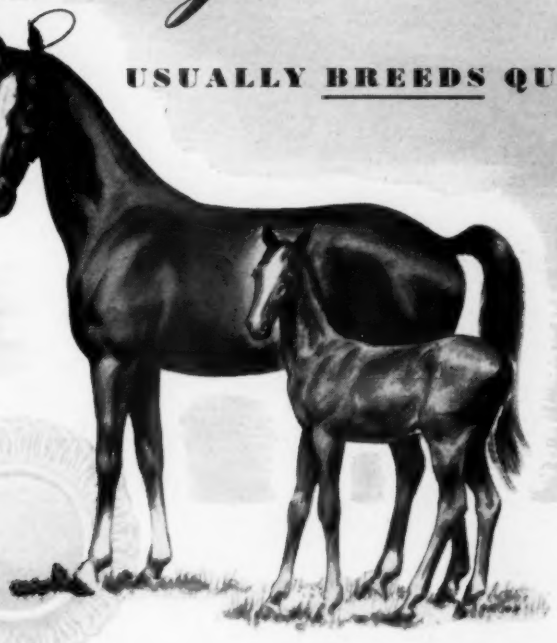

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*__Charles, Vera K.,__ 2039 New Hampshire Avenue, N.W., Washington 9, D. C.

Chaudhuri, S. P. Ray, Indian Agricultural Research Institute, New Delhi, India.

Chen, S. M., c/o Dr. K. W. Wegner, 105 Winona Street, Northfield, Minnesota.

Chen, S. T., National University of Shantung, Tsingtao, China.

Cheo, C. C., Department of Plant Pathology, Tsing-Hua University, Peiping, China.

Cherewick, W. J., Dominion Laboratory of Plant Pathology, University of Manitoba, Fort Garry, Manitoba, Canada.

Chester, Kenneth S., Department of Botany, Oklahoma A. & M. College, Stillwater, Oklahoma.

Childs, James F. L., U. S. Subtropical Fruit Station, 415 N. Parramore Street, Orlando, Florida.

Childs, Leroy, Box 89, Hood River, Oregon.

Chilton, St. John P., Department of Botany, Louisiana State University, University, Louisiana.

Chitwood, B. G., Plant Industry Station, Beltsville, Maryland.

Chiu, Wei-Fan, Plant Pathology Department, National Tsing-Hua University, Peiping, China.

Chivers, A. H., Dartmouth College, Hanover, New Hampshire.

Christensen, Clyde M., Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

Christensen, J. J., Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

Christie, Jesse R., Central Florida Experiment Station, Sanford, Florida.

Christoff, Alexander, Krasno Selo near Sofia, Bulgaria.

*__Chupp, Charles,__ Department of Plant Pathology, Cornell University, Ithaca, New York.

Ciccarone, Antonio, Departamento de Fitopatología, Apartado 669, Maracay, Venezuela.

Ciferri, Raffaele, Casella Postale 165, Pavia, Italy.

Clapper, Russell B., Division of Forest Pathology, Plant Industry Station, Beltsville, Maryland.

*__Clara, Feliciano M.,__ c/o Bureau of Plant Industry, 692 San Andres Street, Malate, Manila, Philippine Islands.

Clark, Everett B., Eastern Breeding Station, Clarktown, Milford, Connecticut.

Clayton, Carlyle N., Botany Department, North Carolina State College, Raleigh, North Carolina.

Clayton, E. E., Plant Industry Station, Beltsville, Maryland.

Clulo, Genevieve B., West Virginia University, Morgantown, West Virginia.

Clum, H. H., 40 Smith Street, Chappaqua, Westchester County, New York.

Cochran, George W., Utah State Agricultural College, Logan, Utah.

Cochran, Lloyd C., Citrus Experiment Station, Riverside, California.

Cochrane, Vincent W., Department of Biology, Wesleyan University, Middletown, Connecticut.

Coe, Donald M., California Department of Agriculture, State Office Building 1, Sacramento, California.

Coelho, Mario, Escola S. Agricultura, Recife, Pernambuco, Brazil.

Cohen, Mortimer, Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

Cohen, S. I., Gallowhur Chemical Corporation, 801 Second Avenue, New York 17, New York.

Cohill, Roger W., Miller Chemical & Fertilizer Corp., 1000 S. Caroline Street, Baltimore, Maryland.

Cole, J. R., Box 60, Albany, Georgia.

Coleman, Madeline F., 3003 Dancy St., Austin, Texas.

Colley, Reginald H., 8 Winthrop Place, Maplewood, New Jersey.

Compton, L. E., Agricultural Experiment Station, Purdue University, Lafayette, Indiana.

Conant, George H., Ripon, Wisconsin.

Conners, I. L., Central Experimental Farm, Ottawa, Canada.

Conover, Robert A., Sub-Tropical Experiment Station, Route 2, Box 508, Homestead, Florida.

Cook, Harold T., Virginia Truck Experiment Station, Norfolk, Virginia.

*__Cook, Mel T.,__ 255 Barnard Road, Larchmont, New York.

Cook, W. R. I., University College of South Wales, Newport Road, Cardiff, Wales.

Cooley, J. S., Division of Fruit and Vegetable Crops and Diseases, Plant Industry Station, Beltsville, Maryland.

Coombs, R. C., Henniker, New Hampshire.

*__Coons, G. H.__, Plant Industry Station, Beltsville, Maryland.

Cooper, William E., Upper Coastal Plain Station, Rocky Mount, North Carolina.

Cormack, M. W., Dominion Laboratory of Plant Pathology, University of Alberta, Edmonton, Alberta, Canada.

Corp, Helen Virginia, Plant Pathology Department, University of Maryland, College Park, Maryland.

*__Cotter, Ralph U.__, Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

Courtney, W. D., U. S. Department of Agriculture, Nematology, Western Washington Experiment Station, Puyallup, Washington.

Cowan, E. Karl, Box 190, Interlaken, New York.

Cowart, Linton E., Botany Department, Louisiana State University, Baton Rouge, Louisiana.

Cox, Carroll E., Department of Botany, University of Maryland, College Park, Maryland.

Cox, John A., Forestry Building, Louisiana State University, Baton Rouge, Louisiana.

Cox, Robert S., Box 5397, State College Station, Raleigh, North Carolina.

Craig, F. Waldo, State Department of Agriculture, Charleston, West Virginia.

Craigie, J. H., Division of Botany, Central Experimental Farm, Ottawa, Canada.

Crall, James M., Botany Department, University of Missouri, Columbia, Missouri.

Cralley, E. M., Department of Plant Pathology, University of Arkansas, Fayetteville, Arkansas.

Crandall, Bowen S., 927 W. Main St., Tupelo, Mississippi.

Creager, D. B., R.F.D. 2, St. Anne, Illinois.

Crittenden, Henry W., Botany Department, Ohio State University, Columbus 10, Ohio.

Crocker, William, Boyce Thompson Institute for Plant Research, Yonkers 3, New York.

Crosier, Willard F., Division of Seed Investigations, New York State Agricultural Experiment Station, Geneva, New York.

Crumb, S. E., Jr., Entomology Department, Oregon State College, Corvallis, Oregon.

Cummins, George B., Botany Department, Purdue University, Lafayette, Indiana.

Cunniff, Hilda S., 404 South St., Key West, Florida.

Cunningham, H. S., Long Island Vegetable Research Farm, New York State Experiment Station, Riverhead, New York.

Daines, Robert H., Department of Plant Pathology, New Jersey Agricultural Experiment Station, New Brunswick, New Jersey.

Dallimore, C. E., Branch Agricultural Experiment Station, Aberdeen, Idaho.

Daly, Joseph M., Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

Dana, Bliss F., Department of Botany, Oregon State College, Corvallis, Oregon.

Dantas, Bento, Instituto Agronômico do Noter, Belem, Pará, Brazil.

Darker, Grant D., Ben Venue Laboratories, Inc., Bedford, Ohio.

Darley, Ellis, F., Department of Botany and Plant Pathology, Colorado A. & M. College, Ft. Collins, Colorado.

Darling, H. M., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

Das Gupa, S. N., Department of Botany, University of Lucknow, Lucknow, U. P., India.

Davidson, R. S., Department of Botany and Plant Pathology, Ohio Agricultural Experiment Station, Wooster, Ohio.

Davidson, Ross W., Plant Industry Station, Beltsville, Maryland.

Davidson, T. R., Dominion Laboratory of Plant Pathology, University of Alberta, Edmonton, Alberta, Canada.

Davies, Fred R., c/o Rohm & Haas Co., Bristol, Pennsylvania.

Davis, B. H., Department of Plant Pathology, Agricultural Experiment Station, New Brunswick, New Jersey.

Davis, David, 909 S. First Street, Champaign, Illinois.

Davis, Glen N., Division of Truck Crops, California Agricultural Experiment Station, Davis, California.

Davis, Spencer H., Jr., College Farm, Rutgers University, New Brunswick, New Jersey.

Davis, V. H., c/o Promotion Department, Farm Bureau Cooperative Association, 246 North High Street, Columbus, Ohio.

Davis, William C., c/o R. H. Wyche, Texas Agricultural Experiment Sta-

tion, Sub-Station No. 4, Beaumont, Texas.

Davy, Roger H., Box 181, Cadiz, Kentucky.

Dean, Horace S., 211 George Mason Road, Falls Church, Virginia.

Dean, Leslie L., Purdue Agricultural Experiment Station, Lafayette, Indiana.

deBruyn, Helena L. G., Hinkeloordsche Weg 5, Wageningen, Holland.

Decker, Phares, Department of Plant Pathology, Florida Agricultural Experiment Station, Gainesville, Florida.

Delwiche, Edmund D., 1140 Clark Way, San Jose 10, California.

Demaree, J. B., Plant Industry Station, Beltsville, Maryland.

Demiriz, Ahmet Husna, Ciftehavuzlar 72, Erenkoy, Istanbul, Turkey.

Denny, F. E., Box 20, Bristol, Vermont.

de Ong, E. R., 926 Stannage Avenue, Albany 6, California.

de Ropp, R. S., New York Botanical Gardens, Bronx Park (Fordham P. O.), New York 58, New York.

Desjardins, Paul R., 107 Hilgard Hall, University of California, Berkeley 4, California.

Deslandes, Josué, Instituto Agronomico do Sul, Pelotas, Rio Grande do Sul, Brazil.

Desrosiers, Russell, Box 13, Stafford, Connecticut.

Dessureaux, Lionel, Experimental Station, Ste-Anne de la Pocatiere, Quebec, Canada.

Dezeeuw, Donald J., Botany Department, Michigan State College, East Lansing, Michigan.

Diachun, Stephen, Agricultural Experiment Station, Lexington 29, Kentucky.

Dickinson, Sydney, School of Agriculture, Cambridge, England.

*__Dickson, B. T.__, Chief, Division of Plant Industry, C. S. I. R. Box 109, Canberra, F. C. T., Australia.

Dickson, James G., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

Dickson, Warrick J., Botany Department, Louisiana State University, Baton Rouge 3, Louisiana.

Diehl, William W., 1512 N. McKinley Road, Arlington, Virginia.

Dietz, S. M., Botany Department, Oregon State College, Corvallis, Oregon.

Diller, Jesse D., 40 N. Highland Street, Arlington, Virginia.

Dimitman, Jerome E., 6727 Cedar Street, Huntington Park, California.

Dimock, A. W., Department of Plant Pathology, Cornell University, Ithaca, New York.

Dimond, Albert E., Connecticut Agricultural Experiment Station, P.O. Drawer 1106, New Haven 4, Connecticut.

Dippenaar, B. J., Agricultural Research Institute, c/o Universiteit Van Pretoria, Pretoria, Union of South Africa.

*__Dobbins, Raymond A.__, Department of Biology, Ohio Northern University, Ada, Ohio.

Dodge, B. O., New York Botanical Garden, New York 58, New York.

*__Dodge, Carroll W.__, Missouri Botanical Garden, 2315 Tower Grove Avenue, St. Louis, Missouri.

Dolan, Desmond, Department of Horticulture, Rhode Island State College, Kingston, Rhode Island.

Doolittle, S. P., Plant Industry Station, Beltsville, Maryland.

Doran, William L., 148 Lincoln Avenue, Amherst, Massachusetts.

Dosdall, Louise T., Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

Downie, Andrew R., Beet Seed Operations, American Crystal Sugar Co., Rocky Ford, Colorado.

Drayton, F. L., Division of Botany, Central Experimental Farm, Ottawa, Canada.

Drechsler, Charles, Division of Fruit and Vegetable Crops and Diseases, Plant Industry Station, Beltsville, Maryland.

DuBuy, Herman G., 5013 Hollywood Road, Berwyn, Maryland.

Ducharme, Ernest, Citrus Experiment Station, Lake Alfred, Florida.

Dufour, M. F., The Micronizer Company, Moorestown, New Jersey.

Dufrenoy, Jean, 2282 Union Street, Berkeley 4, California.

Duggar, B. M., c/o Lederle Laboratories, Inc., Pearl River, New York.

Dulaney, Eugene L., 171 Maple Avenue, Rahway, New Jersey.

Dunckelman, P. H., 942 N. 6th Street, Baton Rouge, Louisiana.

Dundas, Bjarne, Milpitas, California.

Dunegan, John C., Plant Industry Station, Beltsville, Maryland.

Dunlap, Albert A., Division of Plant Pathology, Agricultural Experiment Station, College Station, Texas.

Durand, Kenneth H., 405 N. Wilson, Modesto, California.

Durrell, L. W., Botany Department, Colorado Agricultural College, Fort Collins, Colorado.

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Eddins, Auther H., Hastings, Florida.

*__Edgerton, C. W.__, Department of Botany, Louisiana State University, University, Louisiana.

*__Edson, H. A.__, 3810 Fourth Street, N.W., Washington 11, D. C.

Ehrlich, John, Research Laboratories, Parke Davis & Co., Detroit 32, Michigan.

Eide, Carl J., Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

El-Helaly, A. F., Farouk 1 University, Alexandria, Egypt.

Ellett, C. W., Department of Botany, Ohio State University, Columbus 10, Ohio.

Elliott, Edward S., Bruceton Mills, West Virginia.

*__Elliott, Charlotte__, Westgate Lane, Lanham, Maryland.

Ellis, Agnes M., Division of Forest Pathology, Plant Industry Station, Beltsville, Maryland.

Ellis, Don E., Plant Pathology Section, Box 5397, North Carolina State College, Raleigh, North Carolina.

Elmer, Otto H., Department of Botany and Plant Pathology, Kansas State College, Manhattan, Kansas.

Emerson, Frank H., Department of Botany, University of Kansas, Lawrence, Kansas.

Emmons, Maryhelen, Department of Plant Pathology, University of Arizona, Tucson, Arizona.

Enge, Robert G., 1510 Delmont Court, Urbana, Illinois.

English, W. Harley, Division of Plant Pathology, University of California, Davis, California.

Epps, James M., West Tennessee Experiment Station, Jackson, Tennessee.

Epps, William M., Truck Experiment Station, Box 337, Charleston, South Carolina.

Ergle, David R., Agricultural Experiment Station, College Station, Texas.

Ernest, Carol T., Botany Department, Louisiana State University, Baton Rouge, Louisiana.

Evans, Arlyn W., 2807 Orchard Street, Corvallis, Oregon.

Evans, J. A., 5029 Du Pont Building, Wilmington, Delaware.

Exner, Beatrice, 906 St. Ferdinand St., Baton Rouge, Louisiana.

Ezekiel, Walter N., Navy Department, Bureau of Ordnance, Washington 25, D. C.

Fairchild, S. J., 426 East Cook Street, Santa Maria, California.

Fang, C. T., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

*__Faull, J. H.__, Arnold Arboretum, Harvard University, Jamaica Plain, Massachusetts.

Favret, E. A., Araoz 2361–DTO C, Buenos Aires, RCA, Argentina.

*__Fawcett, Howard S.__, Citrus Experiment Station, Riverside B, California.

Feder, William A., 5494 College Avenue, Oakland, California.

Feichtmeir, Edmund F., General Delivery, Ripon, California.

Feldman, Albert W., Rhode Island Agricultural Experiment Station, Kingston, Rhode Island.

Felix, E. L., Agricultural Experiment Station, University of Tennessee, Knoxville, Tennessee.

Fellows, Hurley, Department of Botany and Plant Pathology, Kansas State College, Manhattan, Kansas.

Felton, Mathias W., Nebraska Certified Potato Growers, Alliance, Nebraska.

Fenne, S. B., Blacksburg, Virginia.

*__Fenner, L. M.__, 18 Liberty Street, Bloomsfield, New Jersey.

Fernow, Karl H., Department of Plant Pathology, Cornell University, Ithaca, New York.

Feustel, William K., 33 Winfield Street, East Norwalk, Connecticut.

*__Fields, W. S.__, Demarest, New Jersey.

Fife, James M., 3201 Pine Street, Riverside, California.

Fink, Harry C., Botany Hall, Iowa State College, Ames, Iowa.

Finley, Arthur M., Botany Department, University of Missouri, Columbia, Missouri.

Fiore, Charles, Prairie View, Illinois.

*__Fischer, George W.__, Department of Plant Pathology, State College of Washington, Pullman, Washington.

Fischetti, Dario L., Paseo Colón 285, Buenos Aires, Argentina.

Fisher, Francine E., Citrus Experiment Station, Lake Alfred, Florida.

Fitchett, Donald J., 301 E. Ashley Street, Jefferson City, Missouri.

Fitzpatrick, H. M., Department of Plant Pathology, Cornell University, Ithaca, New York.

Fitzpatrick, Hugh F., 3951 Everest Street, Arlington, California.

Fletcher, Donald G., 415 McKnight Bldg., Minneapolis 1, Minnesota.
Flor, Harold H., State College Station, Fargo, North Dakota.
Foll, S. C., 797 N. Milwaukee Street, Milwaukee, Wisconsin.
Folsom, Donald, Maine Agricultural Experiment Station, Orono, Maine.
Forbes, I. L., Department of Botany & Plant Pathology, Louisiana State University, University, Louisiana.
Ford, Clifford H., Long Island Agricultural and Technical Institute, Farmingdale, New York.
Forsberg, J. L., 337 Natural Resources Building, Urbana, Illinois.
Foster, A. Alfred, Long Island Agricultural and Technical Institute, Farmingdale, New York.
Foster, Harold H., Department of Botany and Bacteriology, Clemson Agricultural College, Clemson, South Carolina.
Foster, Robert E. II, Department of Plant Pathology, New York Agricultural Experiment Station, Geneva, New York.
Foster, W. R., Department of Agriculture, Parliament Buildings, Victoria, B. C., Canada.
Fowler, Marvin E., Plant Industry Station, Beltsville, Maryland.
Fracker, S. B., Agriculture Research Administration, U. S. Department of Agriculture, Washington 25, D. C.
Frank, Arthur, Ferry-Morse Seed Co., R. F. D. 1, Box 543, Rochester, Minnesota.
Frazier, Norman W., Division of Entomology, University of California, Berkeley 4, California.
*__Freeman, E. M.__, Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.
Freitag, J. H., Division of Entomology, University of California, Berkeley 4, California.
French, Alexander M., Plant Pathology Department, Cornell University, Ithaca, New York.
French, G. T., 1112 State Office Building, Richmond 19, Virginia.
Fridlund, Paul R., Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.
Friedman, Bernard A., U. S. Department of Agriculture, Room 1022, 641 Washington Street, New York 14, New York.
*__Fromme, F. D.__, Office of Experiment Stations, U. S. Department of Agriculture, Washington 25, D. C.
Fulmer, W. L., 505 Boylston Avenue N., Seattle 2, Washington.
*__Fulton, Harry R.__, Plant Industry Station, Beltsville, Maryland.
Fulton, J. P., Department of Plant Pathology, University of Arkansas, Fayetteville, Arkansas.
Fulton, Joseph P., 308 Natural History Building, Urbana, Illinois.
Fulton, Robert W., Horticulture Department, University of Wisconsin, Madison 6, Wisconsin.

Gaines, J. G., Coastal Plain Experiment Station, Tifton, Georgia.
Gallegly, M. E., Jr., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.
Garces O., Carlos, Palace 58–59, Medellin, Colombia.
Garcia Rada, German, La Molina Agricultural Experiment Station, La Molina, Peru.
Gardner, Leo R., California Spray Chemical Corp., Richmond, California.
*__Gardner, Max W.__, Division of Plant Pathology, University of California, Berkeley 4, California.
Garren, Kenneth H., Department of Botany, A. P. I., Auburn, Alabama.
Garriss, Howard R., State College Station, Raleigh, North Carolina.
Gaskill, John O., Box 532, Fort Collins, Colorado.
Gaulet, Pauline L., Brooklyn Botanic Garden, 1000 Washington Avenue, Brooklyn 25, New York.
Gäumann, Ernst, Eidg. Technische Hochschule, Universitätsstrasse, Zurich, Switzerland.
Geise, Fred W., American Can Company, 104 S. Michigan Avenue, Chicago 3, Illinois.
Gemmell, Louis G., 237 W. Union Avenue, Bound Brook, New Jersey.
Gerdemann, J. W., 1405 Josephine Street, Berkeley 3, California.
Gibler, John W., Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.
Gibson, Frederick, Superior, Arizona.
*__Giddings, N. J.__, Box 31, Riverside, California.
Gilbert, E. M., Biology Building, University of Wisconsin, Madison 6, Wisconsin.
Gilgut, C. J., Waltham Field Station, Waltham, Massachusetts.
Gill, Denzell L., Field Laboratory, Springhill, Alabama.
*__Gilman, Joseph C.__, 518 Ash Avenue, Ames, Iowa.
Gilmer, Ralph, Box 304, Morgan Hill, California.
Gilmer, Robert M., 2514 E. Johnson Street, Madison, Wisconsin.

Gloyer, W. O., 106 Lyceum Street, Geneva, New York.

Godfrey, G. H., Texas Agricultural Experiment Substation 15, Weslaco, Texas.

Goidanich, Gabriel, via S. Susanna, Stazione di Patologia Vegetale, Roma, Italy.

Golding, Pearl, 418½ Second Street, Davis, California.

Goldsworthy, M. C., Plant Industry Station, Beltsville, Maryland.

Gooch, F. S., Southwestern Louisiana Institute, Lafayette, Louisiana.

Good, H. M., Department of Botany, University of Toronto, Toronto 5, Canada.

Goodman, J. J., Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

Gordon, Dorothy R., 403 East 8th Street, Loveland, Colorado.

Gordon, Fred M., Nassau County Farm Bureau, Mineola, New York.

Gordon, W. L., Dominion Rust Research Laboratory, University of Manitoba, Winnipeg, Manitoba, Canada.

Gorenz, August M., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

Goss, R. W., Department of Plant Pathology, College of Agriculture, Lincoln 1, Nebraska.

Gottlieb, David, Department of Horticulture, University of Illinois, Urbana, Illinois.

Goto, Shosuke, Division of Plant Pathology and Botany, University Farm, St. Paul 1, Minnesota.

Gotz, Mathilde Bensaude, 410 Riverside Drive, New York 25, New York.

Gould, C. J., Western Washington Experiment Station, Puyallup, Washington.

Graham, J. H., Box 5397, North Carolina State College, Raleigh, North Carolina.

Graham, Richard W., 155 Forest Street, Veterans Village, Ft. Collins, Colorado.

Graham, Thomas W., Pee Dee Experiment Station, Florence, South Carolina.

Grainger, John, Department of Plant Pathology, The West of Scotland Agricultural College, Auchincruive by Ayr, Scotland.

Granovsky, Alexander A., Division of Entomology, University Farm, St. Paul 1, Minnesota.

Grant, Theodore J., c/o Consulate General, São Paulo, Brazil.

Gratz, L. O., Agricultural Experiment Station, Gainesville, Florida.

Gravatt, Annie R., Division of Information, Plant Industry Station, Beltsville, Maryland.

Gravatt, G. Flippo, Plant Industry Station, Beltsville, Maryland.

*__Graves, Arthur H.__, 255 S. Main Street, Wallingford, Connecticut.

Greaney, F. J., Northwest Line Elevators, Grain Exchange Building, Winnipeg, Manitoba, Canada.

Greene, H. C., Biology Building, University of Wisconsin, Madison 6, Wisconsin.

Greenleaf, Walter, Alabama Experiment Station, Auburn, Alabama.

Gregory, Chas. T., 229 W. Jewel Avenue, Kirkwood 22, Missouri.

Gries, George A., Agricultural Experiment Station, Purdue University, Lafayette, Indiana.

Griffith, R. B., 6127 Magnolia Avenue, Riverside, California.

Grogan, Raymond G., Department of Plant Pathology, University of California, Davis, California.

Groves, A. B., 1415 Greystone Terrace, Winchester, Virginia.

Gruenhagen, R. H., Biochemical Research Laboratory, Dow Chemical Co., Midland, Michigan.

Guba, E. F., Waltham Field Station, 240 Beaver Street, Waltham, Massachusetts.

Guiscafre-Arrillaga, J., c/o Department of Plant Pathology, Louisiana State University, Baton Rouge, Louisiana.

Gupta, B. M., Department of Biology, Phipps Conservatory, University of Pittsburgh, Pittsburgh, Pennsylvania.

Guterman, Carl E. F., Roberts Hall, Cornell University, Ithaca, New York.

Guy, H. G., Research Department, Koppers Co., Pittsburgh 19, Pennsylvania.

Haas, Carstens Y., Box 388, Philadelphia 5, Pennsylvania.

Haasis, Frank A., Box 786, Babylon, New York.

Hadden, S. J., Coker's Pedigreed Seed Co., Hartsville, South Carolina.

Haenseler, C. M., Agricultural Experiment Station, New Brunswick, New Jersey.

Hafstad, George E., 616 Mulberry Street, Macon, Georgia.

Hagborg, W. A. F., Dominion Laboratory of Plant Pathology, Manitoba Agricultural College, Winnipeg, Manitoba, Canada.

Hagedorn, Donald J., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

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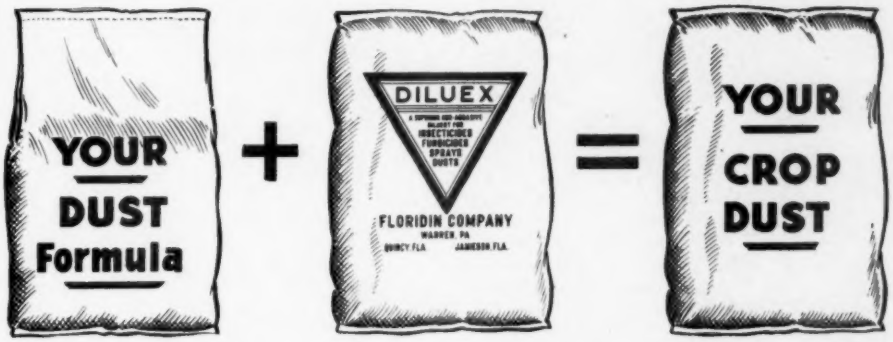

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Hall, Dennis H., Utah State Agricultural College, Logan, Utah.

Hamilton, James M., Experiment Station, Geneva, New York.

Hampton, Jack E., Department of Plant Pathology, University of Arizona, Tucson, Arizona.

Hanna, W. F., Dominion Rust Research Laboratory, University of Manitoba, Winnipeg, Manitoba, Canada.

Hansen, H. N., Division of Plant Pathology, University of California, Berkeley 4, California.

Hansing, Earl D., Department of Botany and Plant Pathology, Kansas State College, Manhattan, Kansas.

Hanson, Earle W., Plant Pathology Division, University of Wisconsin, Madison 6, Wisconsin.

Hardh, K., Kuusitie 15 B54, Helsinke, Finland.

Hardison, John R., Botany Department, Oregon Agricultural Experiment Station, Corvallis, Oregon.

Hare, Woodrow W., Department of Plant Pathology, Mississippi Agricultural Experiment Station, State College, Mississippi.

Harrar, J. G., Calle Viena 26, Mexico, D.F., Mexico.

Harris, Marion R., Extension Service, Washington State College, Pullman, Washington.

Harrison, A. L., Tomato Disease Laboratory, Yoakum, Texas.

Harrison, Charles, 2318 N. 38th Street, Seattle 3, Washington.

Harrison, George J., U. S. Cotton Field Station, Shafter, California.

Harrison, Kenneth A., Kentville, Nova Scotia, Canada.

Harry, John B., Boyce Thompson Institute, Yonkers 3, New York.

Hart, Helen, Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

*__Hartley, Carl,__ Plant Industry Station, Beltsville, Maryland.

Hartman, John D., Department of Horticulture, Purdue University, Lafayette, Indiana.

Hartzell, Albert, Boyce Thompson Institute, Yonkers 3, New York.

Harvey, James V., 2800 South 20th Street, Philadelphia 45, Pennsylvania.

Hasanain, Syed Zulfiquarul, University Farm, St. Paul 1, Minnesota.

*__Haskell, R. J.,__ Extension Service, U. S. Department of Agriculture, Washington 25, D. C.

Hassan, Hussein H., Department of Horticulture, University of Illinois, Urbana, Illinois.

Hassan, S. F., Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

Hatfield, W. C., American Can Company, 111 Sutter Street, San Francisco 4, California.

Hauser, Richard S., Department of Botany and Plant Pathology, Michigan State College, East Lansing, Michigan.

Haymaker, H. H., Department of Botany and Plant Pathology, Kansas State College, Manhattan, Kansas.

Heald, F. D., 312 Howard Street, Pullman, Washington.

Hebert, Teddy T., Botany Department, North Carolina State College, Raleigh, North Carolina.

Hedges, Florence, c/o Dr. John Stevenson, Plant Industry Station, Beltsville, Maryland.

Heggestad, Howard E., Tobacco Experiment Station, Greeneville, Tennessee.

Heimbeck, Louise, Brosve, Levanger, Norway.

Hemingway, W. C., Route 3, Twin Falls, Idaho.

*__Henderson, R. G.,__ Department of Botany and Plant Pathology, Agricultural Experiment Station, Blacksburg, Virginia.

Hendrix, J. Walter, Hawaii Agricultural Experiment Station, University of Hawaii, Honolulu, Hawaii.

Henker, Sergio Tartakowsky, Box 4647, Santiago, Chile.

Henry, A. W., University of Alberta, Edmonton, Alberta, Canada.

Henry, Berch, Ashe Forest Nursery, Brooklyn, Mississippi.

Henson, Lawrence, Department of Agronomy, Kentucky Agricultural Experiment Station, Lexington 29, Kentucky.

Hepting, George H., U. S. Division of Forest Pathology, 223 Federal Building, Asheville, North Carolina.

*__Hesler, L. R.,__ Department of Botany, University of Tennessee, Knoxville, Tennessee.

Hesseltine, C. W., Mountain View Gardens, Pearl River, New York.

Heuberger, John W., Department of Plant Pathology, University of Delaware, Newark, Delaware.

Hewitt, J. Lee, 877 Canterbury Road, Sacramento 15, California.

Hewitt, William B., Division of Plant Pathology, University Farm, Davis, California.

Heyne, Elmer G., Department of Agronomy, Kansas State College, Manhattan, Kansas.

*__Higgins, B. B.__, Department of Botany, Georgia Experiment Station, Experiment, Georgia.

Hilborn, Merle T., Agricultural Experiment Station, Orono, Maine.

Hildebrand, A. A., Harrow, Ontario, Canada.

Hildebrand, Earl M., Biology Department, Texas A. & M., College Station, Texas.

Hildebrandt, Albert C., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

Hill, John P., 300 Elizabeth Street, Redlands, California.

Hingorani, M. K., Lodi Road Colony, Block 5, House 583, New Delhi, India.

Hirschhorn, Elisa, Estacion Experimental, Central Castelav, F. C. O., Argentina.

Hirt, Ray R., New York State College of Forestry, Syracuse 1, New York.

Hitchner, L. S., 285 Madison Avenue, New York 17, New York.

Hockey, J. F., Kentville, N. S., Canada.

Hodges, F. Allen, Food & Drug Administration, Federal Security Agency, Washington 25, D. C.

Hoerner, G. R., Oregon State College, Corvallis, Oregon.

Holbert, J. R., Box 828, Funk Bros. Seed Co., Bloomington, Illinois.

Holdeman, Quintin L., Botany Department, Louisiana State University, Baton Rouge 3, Louisiana.

Hollis, John P., Jr., Department of Plant Pathology, Connecticut Agricultural Experiment Station, New Haven 4, Connecticut.

Holmes, Francis O., Rockefeller Institute for Medical Research, Princeton, New Jersey.

Holton, C. S., Department of Plant Pathology, State College of Washington, Pullman, Washington.

Honey, Edwin E., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

Hooker, William J., Section of Botany and Plant Pathology, Iowa Agricultural Experiment Station, Ames, Iowa.

Hoppe, Paul E., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

Hopperstead, S. L., B. F. Goodrich Chemical Co., 324 Rose Building, Cleveland, Ohio.

Horn, Norman L., Department of Plant Pathology, University of Maryland, College Park, Maryland.

Horsey, Robert E., Sindar Corporation, 330 W. 42nd Street, New York 18, New York.

*__Horsfall, James G.__, Agricultural Experiment Station, New Haven 4, Connecticut.

Hotson, J. W., Johnson Hall, University of Washington, Seattle 5, Washington.

Hough, L. Frederic, Department of Horticulture, Rutgers University, Agricultural Experiment Station, New Brunswick, New Jersey.

Houston, Byron R., College of Agriculture, Davis, California.

Howard, Frank L., Department of Botany, Rhode Island State College, Kingston, Rhode Island.

Howard, Grace E., Botany Department, Wellesley College, Wellesley, Massachusetts.

Howitt, J. E., Ontario Agricultural College, Guelph, Ont., Canada.

Howland, Joseph E., Meredith Publishing Co., Des Moines, Iowa.

Hoyman, William G., Department of Plant Pathology, North Dakota State College, Fargo, North Dakota.

Hsia, Yu-Tien, Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

Huffington, Jesse M., Chef Boy-Ar-Dee Quality Foods, Inc., Biglerville, Pennsylvania.

Humphrey, C. J., 150 S. Gardner Street, Los Angeles 36, California.

*__Humphrey, H. B.__, Box 14, Cosmos Club, Washington 5, D. C.

Hungerford, Chas. W., Department of Plant Pathology, University of Idaho, Moscow, Idaho.

Hunt, N. Rex, Felton, California.

*__Hunter, Herman A.__, 405 Magnolia Avenue, Frederick, Maryland.

Hurt, Robert H., Piedmont Field Laboratory, University of Virginia, Charlottesville, Virginia.

*__Hutchins, Lee M.__, Plant Industry Station, Beltsville, Maryland.

Hwang, L., College of Agriculture, National Kwangsi University, Kweilin, Kwangsi, China.

Hyre, Russel A., Department of Plant Pathology, University of Delaware, Newark, Delaware.

Ibrahim, Ishmail A., Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

Imle, E. P., U. S. Department of Agriculture, Cooperative Rubber Plant Field Station, Turrialba, Costa Rica.

Inman, F. G., 24 Avery Avenue, Buffalo, New York.

Irons, Frank, Box 107, Station E, Toledo, Ohio.

Irving, George W., Jr., Bureau of Agricultural and Industrial Chemistry, Department of Agriculture, South Building, Washington 25, D. C.

Isaakides, Constantin A., Ministere de l'Agriculture, Athens, Greece.

Ivanoff, S. S., Department of Plant Pathology, Mississippi Agricultural Experiment Station, State College, Mississippi.

*__Jackson, H. S.__, Department of Botany, University of Toronto, Toronto, Ontario, Canada.

Jackson, Lyle W. R., Forestry Department, University of Georgia, Athens, Georgia.

Jacques, J. Emile, Montreal Botanical Garden, Montreal, Canada.

Jamalainen, E. A., Finland Agricultural Experiment Station, Tikkurila, Finland (Suomi).

James, W. S., Micronizer Processing Co., Moorestown, New Jersey.

Janson, Blair F., Department of Botany, Ohio State University, Columbus 10, Ohio.

Jauch, Clotilde, Freyre 2175, Buenos Aires, Argentina.

Jeffers, Walter F., 6824 Pine Way, College Heights, Hyattsville, Maryland.

*__Jehle, R. A.__, Extension Service, Maryland State Agricultural College, College Park, Maryland.

Jenkins, Anna E., 2310 Connecticut Avenue, N. W., Washington 8, D. C.

Jenkins, Wilbert A., Virginia Tobacco Research Station, Chatham, Virginia.

Jensen, D. D., 112 Agriculture Hall, University of California, Berkeley, California.

Jensen, James H., Atomic Energy Commission, 1901 Constitution Avenue, Washington 25, D. C.

Johann, Helen, 1320 Spring Street, Madison, Wisconsin.

*__Johnson, Aaron G.__, Plant Industry Station, Beltsville, Maryland.

Johnson, Conrad C., c/o Innis Speiden & Co., 117 Liberty Street, New York 6, New York.

*__Johnson, Edward M.__, Agricultural Experiment Station, Lexington 29, Kentucky.

Johnson, Folke, Western Washington Experiment Station, Puyallup, Washington.

Johnson, Herbert G., c/o Yoder Bros., Box 230, Barberton, Ohio.

Johnson, Howard B., Windermere, Florida.

Johnson, James, Horticulture Building, University of Wisconsin, Madison 6, Wisconsin.

Johnson, Nels J., 1212 Elmwood Avenue, Evanston, Illinois.

Johnson, T., Dominion Rust Research Laboratory, University of Manitoba, Winnipeg, Manitoba, Canada.

Johnson, T. C., Box 263, Lancaster, Pennsylvania.

Johnson, C. O., Department of Botany and Plant Pathology, Kansas State College, Manhattan, Kansas.

*__Jones, Fred R.__, Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

*__Jones, L. K.__, Box 893, Hilo, Hawaii.

Jones, S. G., The University, Glasgow, Scotland.

Jørstad, Ivar, Botanical Museum, Oslo, Norway.

Josephson, Leonard M., Agronomy Department, Kentucky Agricultural Experiment Station, Lexington 29, Kentucky.

Jucksch, Harry H., 2019 Yolo Avenue, Berkeley 7, California.

Keener, Paul D., Department of Plant Pathology, College of Agriculture and Experiment Station, University of Arizona, Tucson, Arizona.

Keil, Harry L., Biological Laboratories, Rohm & Haas Co., Bristol, Pennsylvania.

*__Keitt, G. W.__, Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

Kelman, Arthur, Plant Pathology Section, North Carolina State College, Raleigh, North Carolina.

*__Kendrick, J. B.__, Division of Plant Pathology, University Farm, Davis, California.

Kendrick, James B., Jr., Division of Plant Pathology, Citrus Experiment Station, Riverside, California.

Kenknight, Glenn, U. S. Horticulture Field Laboratory, Fort Valley, Georgia.

Kennelty, Ray, Room 601–A, Custom House, Philadelphia 6, Pennsylvania.

Kent, Ellis C., 984 Spruce Street, Salem, Oregon.

Kent, G. C., Department of Plant Pathology, Cornell University, Ithaca, New York.

Kephart, Joyce E., Fruit and Vegetable Division, Plant Industry Station, Beltsville, Maryland.

*__Kern, F. D.__, Pennsylvania State College, State College, Pennsylvania.

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Kernkamp, M. F., Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

Kevorkian, Arthur G., Atkins Garden and Research Laboratory (Soledad), Cienfuegos, Cuba.

Khan, Abdul Hamid, 107 Hilgard Hall, University of California, Berkeley, California.

Khan, Inam U., Citrus Experiment Station, Riverside, California.

Kienholz, J. R., U. S. Fruit Disease Laboratory, P. O. Box 89, Hood River, Oregon.

*__Kightlinger, Clifford V.__, Stockbridge Hall, Massachusetts State College, Amherst, Massachusetts.

Kimney, J. W., 720 Appraisers Building, 630 Sansome Street, San Francisco 11, California.

Kincaid, Randall R., Quincy, Florida.

King, Claude, Kansas State College, Manhattan, Kansas.

King, John W., Morgan State College, Baltimore, Maryland.

King, Thomas H., University Farm, St. Paul 1, Minnesota.

Kingsolver, Charles H., Botany Department, University of Missouri, Columbia, Missouri.

*__Kirby, R. S.__, Department of Botany, Pennsylvania State College, State College, Pennsylvania.

Kirkpatrick, Hugh C., Plant Pathology Department, Cornell University, Ithaca, New York.

Kitchen, Edward M., Pacific Coast Borax Co., 51 Madison Avenue, New York 10, New York.

Klein, Richard M., Department of Botany, University of Chicago, Chicago 37, Illinois.

Klotz, L. J., 4624 Olivewood Avenue, Riverside, California.

Knorr, L. Carl, Citrus Experiment Station, University of Florida, Lake Alfred, Florida.

Koch, Karl, Box 728, Presque Isle, Maine.

Koehler, Benjamin, Department of Agronomy, University of Illinois, Urbana, Illinois.

Kolk, Laura A., 416 Ocean Avenue, Brooklyn, New York.

Kommedahl, Thor, Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

Kopf, Kenneth, Hawaiian Pineapple Co., Honolulu 1, Hawaii.

Koshy, T. A., 105 E. Patterson Avenue, Columbus 2, Ohio.

Kotila, J. E., Plant Industry Station, Beltsville, Maryland.

Kramer, Moyses, Instituto Biológico, Caixa Postal 2821, São Paulo, Brazil.

Kreitlow, Kermit W., U. S. Regional Pasture Research Laboratory, State College, Pennsylvania.

Kreutzer, William A., Shell Agricultural Laboratory, P. O. Box 1531, Modesto, California.

Kunkel, L. O., The Rockefeller Institute for Medical Research, Princeton, New Jersey.

Kuntay, Lelahattin, Yukesk Ziraat Enstitusii, Ankara, Turkey.

Kuntz, James, Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

Lachmund, Harry G., Box 422, Carmel, California.

Lackey, Charles F., Box 31, Bureau of Plant Industry, U. S. Department of Agriculture, Riverside, California.

Ladeburg, R. C., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

Lambert, Edmund B., Plant Industry Station, Beltsville, Maryland.

Lancaster, F. R., The Davey Tree Expert Co., Kent, Ohio.

Lane, George H., Department of Botany and Plant Pathology, Colorado State College, Fort Collins, Colorado.

Langdon, R. F., University of Queensland, Brisbane, Australia.

Langford, A. N., Bishop's University, Lennoxville, Quebec, Canada.

Langford, Michael H., Plant Industry Station, Beltsville, Maryland.

Large, John R., Tung Field Laboratory, Box 812, Bogalusa, Louisiana.

Larsh, Howard W., Department of Plant Sciences, University of Oklahoma, Norman, Oklahoma.

Larson, Russell H., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

Latham, Dennis H., Springfield, Tennessee.

Lavitt, Edwin M., Box 89, Rockville, Connecticut.

Lawyer, Lewis O., California Packing Corp., Morgan Hill, California.

Leach, J. G., Department of Plant Pathology, University of West Virginia, Morgantown, West Virginia.

*__Leach, Lysle D.__, Division of Plant Pathology, University Farm, Davis, California.

Lear, Bert, Department of Plant Pathology, Cornell University, Ithaca, New York.

Lebeau, F. J., U. S. Department of Agriculture, Sugar Plants Field Station, Meridian, Mississippi.

Leben, C., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

LeClerg, E. L., Bureau of Budget, 1724 F Street, N.W., Washington 25, D. C.

Lee, Bertrand W., Maunaloa, Molokai, Hawaii.

Lee, Cecil S., Madre Sierra, Compania Agricola Limitada, Bauta, Havana, Cuba.

*__Lee, H. Atherton,__ Director Agricultural Experiment Station, Mayaguez, Porto Rico.

Lefebvre, C. L., Plant Industry Station, Beltsville, Maryland.

Lehenbauer, R. A., 1208 Patrick Ave., Reno, Nevada.

Lehman, S. G., State College of Agriculture and Engineering, Raleigh, North Carolina.

Leria-Esmoris, José, Box 184, Mayaguez, Porto Rico.

Leukel, R. W., Plant Industry Station, Beltsville, Maryland.

Leutritz, John, Jr., Bell Telephone Laboratories Inc., 409 Broad Street, Summit, New Jersey.

Levine, Michael, 100 Halsted Avenue, Yonkers 4, New York.

*__Levine, M. N.__, Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

Lewis, Fred H., Fruit Research Laboratory, Arendtsville, Pennsylvania.

Lewis, Ralph W., Department of Botany, Michigan State College, East Lansing, Michigan.

Leyendecker, Philip J., Jr., State College, New Mexico.

Lightle, Paul C., 2400 Pepper Drive, Concord, California.

Limber, Donald P., 3 Mayfair Road, Morris Plains, New Jersey.

Liming, O. N., 503 Main Street, East Orange, New Jersey.

Lin, Kung-Hsiang, Lingnan University, Canton, China.

Lindgren, Ralph M., U. S. Division of Forest Pathology, 1008 Federal Office Building, New Orleans, Louisiana.

Lindner, R. C., Tree Fruit Experiment Station, Wenatchee, Washington.

*__Linford, M. B.__, Pineapple Experiment Station, P.O. Box 3166, Honolulu, Hawaii.

Ling, Lee, No. 48, Lane 1340, Sinza Road, Shanghai, China.

Ling, Mrs. Ju-Shen Hsu, 1902 C Street N. E., Washington, D. C.

Link, George K. K., Botany Building, University of Chicago, Chicago 37, Illinois.

Linn, M. B., Department of Horticulture, University of Illinois, Urbana, Illinois.

Litzenberger, S. C., Department of Agronomy, University of Florida, Gainesville, Florida.

Livingston, J. E., Plant Pathology Department, College of Agriculture, Lincoln 1, Nebraska.

Llarden-Molina, Mario, Cornell University, Ithaca, New York.

Locke, S. B., Department of Plant Pathology, State College of Washington, Pullman, Washington.

Locke, Warren F., c/o Waller-Franklin Seed Co., Guadalupe, California.

Loegering, William Q., University Farm, St. Paul 1, Minnesota.

Logsdon, Charles E., Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

*__Loh, T. C.__, Division of Agronomy, Agricultural Experiment Station, Shatong, Liuchow, Kwangsi, China.

Lohman, Marion L., Botany Department, University of Hawaii, P.O. Box 18, Honolulu 10, Hawaii.

Lombard, Frances F., Apt. 1, 5301 38th Avenue, Hyattsville, Maryland.

Lorenz, Roland C., Office Foreign Agricultural Relations, U. S. Department of Agriculture, Washington 25, D. C.

Loucks, Kenneth W., Lake Alfred, Florida.

Lownsbery, Benjamin F., Jr., Department of Plant Pathology, Cornell University, Ithaca, New York.

Lowther, C. V., State College of Washington, Pullman, Washington.

Lu, Shih-I, University Farm, Division of Plant Pathology, St. Paul 1, Minnesota.

Lucas, E. H., Department of Horticulture, Michigan State College, East Lansing, Michigan.

Lucas, G. Blanchard, U. S. Department of Agriculture, Tobacco Laboratory, Oxford, North Carolina.

Lund, Walter T., Department of Botany, Oregon State College, Corvallis, Oregon.

*__Lutman, B. F.__, 111 N. Prospect St., Burlington, Vermont.

Luttrell, E. S., Botany Department, University of Missouri, Columbia, Missouri.

Lyerly, P. J., Texas Agricultural Experiment Station, Substation No. 17, Ysleta, Texas.

Lyle, Eldon W., Texas Rose Research Foundation Inc., R. F. D. 1, Box 130, Tyler, Texas.

Lyle, James A., Department of Botany and Plant Pathology, Alabama Agricultural Experiment Station, Auburn, Alabama.

Lynch, Daniel M., 15724 Grandville Street, Detroit, Michigan.

Machacek, John E., Dominion Rust Research Laboratory, Winnipeg, Manitoba, Canada.

Mack, Guilford L., Agricultural Experiment Station, Geneva, New York.

Mackie, W. W., 1050 San Pablo Avenue, c/o Glenn Denny, Albany 6, California.

MacLachlan, J. D., Department of Botany, Ontario Agricultural College, Guelph, Ontario, Canada.

MacLean, Neil Allen, Department of Plant Pathology, State College of Washington, Pullman, Washington.

*__MacMillan, H. G.__, 1725 Fifth Avenue, Los Angeles 6, California.

Mader, E. O., Faribault, Minnesota.

Magie, Robert O., 2906 Ninth Avenue W., Bradenton, Florida.

Magner, J. M., 516 Bacon Avenue, Webster Groves 19, Missouri.

Mahoney, Charles H., 1739 H Street, N.W., Washington 6, D. C.

Mai, William F., Department of Plant Pathology, Cornell University, Ithaca, New York.

*__Mains, E. B.__, Museum Building, University of Michigan, Ann Arbor, Michigan.

Maliot, Jean C., Box 72, Niland, California.

Maneval, W. E., University of Missouri, Columbia, Missouri.

*__Manns, Thomas E.__, 57 W. Park Place, Newark, Delaware.

Maramorosch, Karl, Brooklyn Botanic Garden, 1000 Washington Avenue, Brooklyn 25, New York.

Marchionatto, Juan B., Ministerio de Agricultura de La Nacio, Dirección de Sanidad Vegetal, Buenos Aires, Argentina.

Marsden, David H., Clark Hall, University of Massachusetts, Amherst, Mas.achusetts.

Marshall, Rush P., 360 Prospect Street, New Haven 11, Connecticut.

*__Martin, G. Hamilton, Jr.__, State Road, South Sudbury, Massachusetts.

Martin, G. W., Box 326, Iowa City, Iowa.

Martin, Lawrence F., Southern Regional Research Laboratory, 2100 Robert E. Lee Boulevard, New Orleans 19, Louisiana.

*__Martin, William H.__, Experiment Station, New Brunswick, New Jersey.

Martin, W. J., Botany Department, Louisiana State University, Baton Rouge, Louisiana.

Mason, Curtis L., University of Arkansas, Department of Plant Pathology, Fayetteville, Arkansas.

*__Massey, L. M.__, Department of Plant Pathology, Cornell University, Ithaca, New York.

Mastenbrock, C., Kruisweg 654, Hoofddorp, Holland.

Mather, Stanley M., 2712 Derby Street, Apt. 3, Berkeley, California.

Mathur, Raghubir Sahai, Department of Plant Pathology and Bacteriology, West Virginia University, Morgantown, West Virginia.

Matsumoto, T., Phytopathological Laboratory, Taiwan University, Taipeh, Formosa, China.

Matsuura, Minoru, Box 1882, Hilo, Hawaii.

Matz, Julius, Pedro Miguel, Summit, Canal Zone.

May, Curtis, Plant Industry Station, Beltsville, Maryland.

McCallan, S. E. A., Boyce Thompson Institute for Plant Research, 1086 North Broadway, Yonkers 3, New York.

McClain, R. L., Box 1066, Riverside, California.

McClellan, Wilbur D., Plant Industry Station, Beltsville, Maryland.

McClintock, J. A., Department of Horticulture, Purdue University, Lafayette, Indiana.

McClure, William T., Jr., Westminster, South Carolina.

McColloch, L. P., Plant Industry Station, Beltsville, Maryland.

McConnell, L. J., Bristol Road, Holland, Pennsylvania.

*__McCubbin, W. A.__, 114 E. Glenbrook Road, Bethesda, Maryland.

*__McCulloch, Lucia__, 1326 Euclid Street, Apt. 35, Washington 9, D. C.

McCutcheon, O. D., 131 Eighth Street, Hanford, California.

McDonough, E. S., Biology Department, Marquette University, Milwaukee 3, Wisconsin.

McFadden, E. S., College Station, Texas.

McFarland, Frank T., Department of Botany, University of Kentucky, Lexington 29, Kentucky.

McFarlane, J. S., U. S. Department of Agriculture, P.O. Box 1609, Salinas, California.

McGahen, Joe W., Botany Department, Louisiana State University, Baton Rouge, Louisiana.

McKelvey, J. J., Rockefeller Foundation, Calle Viena 26, Mexico, D. F., Mexico.

McKenzie, Malcolm A., Clark Hall, Massachusetts State College, Amherst, Massachusetts.

*__McKinney, H. H.__, Plant Industry Station, Beltsville, Maryland.

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McLean, Ruth, Department of Bacteriology, University of North Carolina Medical School, Chapel Hill, North Carolina.

McManus, Peter J., Cooperative G.L.F. Soil Building Service, Inc., Terrace Hill, Ithaca, New York.

McNew, George L., Botany Department, Iowa State College, Ames, Iowa.

McWhorter, Frank P., Department of Botany, Oregon State College, Corvallis, Oregon.

Mead, H. W., Dominion Laboratory of Plant Pathology, University of Saskatoon, Saskatoon, Canada.

Mehrlich, F. P., Hawaiian Pineapple Co., Ltd., P.O. Drawer 3380, Honolulu 1, Hawaii.

Mehta, P. R., Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

*__Meinecke, E. P.__, 720 Appraisers Building, 630 Sansome Street, San Francisco 11, California.

*__Meier, Mrs. F. C.__, 6402 Beechwood Drive, Chevy Chase, Maryland.

Meiners, Jack P., Department of Plant Pathology, State College of Washington, Pullman, Washington.

*__Melander, Leonard W.__, Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

Melchers, L. E., Department of Botany and Plant Pathology, Kansas State College, Manhattan, Kansas.

Melendez, Maria de Los Angeles, Calle Viena 26, Mexico, D.F., Mexico.

*__Melhus, I. E.__, Botany Department, Iowa State College, Ames, Iowa.

Menzies, James David, 1000 Memorial Street, Prosser, Washington.

Meredith, Clifford H., Apartado 669, Maracay, Venezuela.

Meuli, Lloyd J., The Great Western Division, Dow Chemical Co., P.O. Box 245, Seal Beach, California.

Michaelson, Merle E., Colorado A. & M. College, Fort Collins, Colorado.

Middleton, John T., Division of Plant Pathology, Citrus Experiment Station, Riverside, California.

Mielke, James L., Forest Pathology, Box 523, Albuquerque, New Mexico.

Milbrath, D. G., 1410 41st Street, Sacramento 16, California.

Milbrath, J. A., Botany Department, Oregon State College, Corvallis, Oregon.

Miles, G. F., Box 66, Holly Oak, Delaware.

Millar, Paul H., War Memorial Building, Little Rock, Arkansas.

Miller, Harold J., Department of Botany, Pennsylvania State College, State College, Pennsylvania.

Miller, H. N., Vegetable Crops Laboratory, Brandenton, Florida.

Miller, Joseph A., 364 Prospect Street, South Orange, New Jersey.

Miller, Julian H., Department of Plant Pathology, University of Georgia, Athens, Georgia.

Miller, J. J., Dominion Laboratory of Plant Pathology, St. Catherines, Canada.

Miller, Lawrence I., Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

Miller, Paul R., Division of Mycology and Disease Survey, Plant Industry Station, Beltsville, Maryland.

Miller, Paul W., Horticulture Department, Oregon State College, Corvallis, Oregon.

Miller, Pierre A., Department of Agriculture, 146 Physics-Biology Building, 405 Hilgard Avenue, Los Angeles 24, California.

Miller, Roy E., 1932 S. W. Water Street, Portland 1, Oregon.

Miller, William Edmund, Department of Plant Pathology and Bacteriology, West Virginia University, Morgantown, West Virginia.

Milliken, Daniel F., Botany Department, University of Missouri, Columbia, Missouri.

Mills, Percy J., Department of Botany, Louisiana State University, University, Louisiana.

Mills, W. D., Department of Plant Pathology, College of Agriculture, Ithaca, New York.

Mills, Wilford R., Department of Botany, Pennsylvania State College, State College, Pennsylvania.

Minz, Gershon, Rehovot Agricultural Experiment Station, Rehovot, Palestine.

Mirhij, John I., American University, Beirut, Lebanon.

Misra, A. P., 29 B. Kutchery Road, Lucknow, U. P., India.

Mitchell, John E., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

*Mix, A. J., Department of Botany, University of Kansas, Lawrence, Kansas.

Mohanty, U. N., Department of Bacteriology, Imperial College of Science and Technology, South Kensington, London S. W. 7, England.

Molina-Llarden, Mario, Plant Pathology Department, Cornell University, Ithaca, New York.

Mook, Paul V., 110 Piper Road, Hamden, Connecticut.

Moore, Elizabeth J., Department of Botany and Bacteriology, University of Texas, Austin 12, Texas.

Moore, E. Leon, Tobacco Experiment Station, Oxford, North Carolina.

Moore, E. S., The Laboratory, Balfour, Cape, South Africa.

*Moore, George T., Missouri Botanical Garden, St. Louis 10, Missouri.

Moore, J. Duain, Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

Moore, John E., Department of Botany, University of Maryland, College Park, Maryland.

Moore, Katherine S., R. F. D. 3, Waverly, Ohio.

Moore, M. B., Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

*Moore, W. D., 501 S. Andrews Avenue, Fort Lauderdale, Florida.

Moorhead, L. O., Route 2, Box 246, St. Helena, California.

Morgan, Omar D., Jr., Stadium Terrace, Court F., Building 63, Apt. 2–C, Champaign, Illinois.

Morland, Harold L., 417 Tiffin Avenue, Sandusky, Ohio.

Morris, H. Elwood, Montana State College, Bozeman, Montana.

Morrison, Lon S., Box 9, Veterans' Village, Stillwater, Oklahoma.

Moses, C. S., Forest Products Laboratory, Madison 6, Wisconsin.

Mujica, Fernando, Box 4647, Santiago, Chile.

Muller, Albert S., Escuela Nacional de Agricultura, Barcena, Villa Nueva, Guatemala.

Mullin, R. S., Truck Experiment Station, Box 2160, Norfolk 1, Virginia.

Muncie, J. H., Department of Botany, Michigan State College, East Lansing, Michigan.

Mundkur, B. B., Indian Agricultural Research Institute, New Delhi, India.

Munn, Mancel T., Agricultural Experiment Station, Geneva, New York.

Munnecke, Donald E., Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

Murakishi, Haruo Harry, Department of Plant Pathology, University of Hawaii, Agricultural Experiment Station, P. O. Box 18, Honolulu 10, Hawaii.

Murphy, Albert, Box 826, Twin Falls, Idaho.

*Murphy, H. C., Botany Department, Iowa State College, Ames, Iowa.

Nagel, Clatus M., Department of Plant Pathology, Agricultural Experiment Station, Brookings, South Dakota.

Nance, Nellie W., Mycology & Disease Survey, Plant Industry Station, Beltsville, Maryland.

Natti, John J., Department of Plant Pathology, Cornell Univer.ity, Ithaca, New York.

Neal, D. C., Agricultural Center, Room 119, University Station, Baton Rouge, Louisiana.

Neergaard, Paul, Phytopathological Laboratory of J. E. Ohlsens Enke, Copenhagen, Denmark.

Nelson, Klayton E., Box 566, Davis, California.

Nelson, Ray, Botany Department, Michigan State College, East Lansing, Michigan.

Nelson, Thomas C., Department of Botany and Plant Pathology, Michigan State College, East Lansing, Michigan.

Nesom, Rivers Y., Southeastern Louisiana College, Hammond, Louisiana.

**Newhall, A. G., Department of Plant Pathology, Cornell University, Ithaca, New York.

Newton, Margaret, 2392 Beach Drive, Victoria, B. C., Canada.

Newton, Nelson H., Box 145, Veterans Village, Stillwater, Oklahoma.

Nichols, Lester P., 314 Ashley Road, Newark, Delaware.

Nichols, Robert A., 2007 Country Club Boulevard, Ames, Iowa.

Nicolaides, George, Plant Quarantine Station, Rosebank, Staten Island, New York.

Niederhauser, John S., c/o Rockefeller Foundation, Calle Viena 26, Mexico, D.F., Mexico.

Nielsen, L. W., Plant Pathology Division, North Carolina State College, Raleigh, North Carolina.

Nienow, Inez, Department of Bacteriology, University of Arizona, Tucson, Arizona.

Nikitin, A. A., 900 Roosevelt Highway, Box 89, College Park, Georgia.

Nixon, Walter H., 98 Zabala Road, Salinas, California.

Nolan, Kenneth G., 1 Cedar West Place, South Norwalk, Connecticut.

Nolla, J. A. B., Mayaguez, Porto Rico.

Nordlinger, Ernest W., 139 N. Clark Street, Chicago 2, Illinois.
Norton, Donald C., Botany and Zoology Building, Ohio State University, Columbus 10, Ohio.
Nugent, Thomas J., Virginia Truck Experiment Station, Norfolk, Virginia.
Nusbaum, Charles J., Edisto Experiment Station, Blackville, South Carolina.
Nutile, Gabriel E., Jr., Box 406, New Haven 2, Connecticut.
Nyland, George, 1403 Maple Street, Pullman, Washington.

Obee, D. J., Boise Junior College, Boise, Idaho.
O'Brien, Harry R., Worthington, Ohio.
O'Brien, Muriel J., 853 President Street, Brooklyn 15, New York.
Offermann, Alfredo M., División de Fitopatología, Paseo Colón 922, Buenos Aires, Argentina.
O'Leary, Keith, Kennett Square, Pennsylvania.
*__Olive, Edgar W.__, 1704 Rodman Street, Hollywood, Florida.
Olson, Conrad J., Box 230, Barberton, Ohio.
Olson, Edward O., Botany Department, Louisiana State University, Baton Rouge, Louisiana.
Orellana, Rodrigro G., Ministerio de Agric. y Cria., Dept. de Fitopatologia, Caracas, E. U., Venezuela.
Orjuela, Juan E., Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.
Ortega Cantero, Benjamin, Dirección General de Agricultura, c/o Oficina de Estudios Especiales, San Jacinto, D.F., Mexico.
Ortega, Ruben, Departamento de Genética, Apartado 660, Maracay, Venezuela.
*__Orton, C. R.__, Science Hall, West Virginia University, Morgantown, West Virginia.
Osborn, Herbert T., 75 Highland Avenue, Los Gatos, California.
*__Osmun, A. V.__, Clark Hall, Massachusetts State College, Amherst, Massachusetts.
Oswald, John W., Division of Plant Pathology, University Farm, Davis, California.
Ou, Shu-Huang, National Agricultural Research Bureau, Ministry of Agriculture and Forestry, Shaolingwei, Nanking, China.
Owen, Emily M., c/o General Aniline and Film Corp., Easton, Pennsylvania.
Owen, John H., Plant Pathology Department, University of Wisconsin, Madison 6, Wisconsin.
Owens, C. E., 303 N. 32nd Street, Corvallis, Oregon.

Paddock, William C., Plant Pathology Department, Cornell University, Ithaca, New York.
Padial, José A., Lugareno 172 Altos, Esquina Pozos Dulces, Havana, Cuba.
Padilla, Ricardo Coronado, Embassy of Mexico, San Salvador, Central America.
Padmanabhan, S. Y., Central Rice Research Institute, P. O. Nayabazar, Cuttack (Orissa), India.
Pady, S. M., McGill University, Dawson College P. O., Quebec, Canada.
Pal, B. P., Indian Agricultural Research Institute, New Delhi, India.
Palchefsky, John, 448 N. Garland Avenue, Youngstown 6, Ohio.
Palmer, Dean F., 402 Civic Center, San Diego 1, California.
Palmer, Hugh C., 505 W. King Avenue, Columbus 1, Ohio.
Palmer, Jeanne Walther, 474 Upper Mountain Avenue, Upper Montclair, New Jersey.
Palmer, Ralph G., 528 Mt. Hope Avenue, Rochester 7, New York.
Palmiter, D. H., Cottage Road, Poughkeepsie, New York.
Pancoast, Harry M., Food Machinery Corp., San Jose 5, California.
Parker, Charles S., 4806 Kenyon Street, Seattle 8, Washington.
Parker, Dorothy, Calle Viena 26, Mexico, D.F., Mexico.
Parker, Kenneth G., Department of Plant Pathology, Cornell University, Ithaca, New York.
Parris, G. K., Box 321, Leesburg, Florida.
Patton, Robert F., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.
Pearson, Oscar H., South West Street, Feeding Hills, Massachusetts.
Peet, Clyde E., Department of Plant Pathology, West Virginia University, Morgantown, West Virginia.
Peklo, Jaroslav, Prague XII, Chodsia 311, Czechoslovakia.
Peltier, George L., Department of Bacteriology, University of Nebraska, Lincoln 8, Nebraska.
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Peterson, Allan G., Division of Entomology, University Farm, St. Paul 1, Minnesota.
Peterson, L. C., Department of Plant Pathology, Cornell University, Ithaca, New York.
Peterson, Paul D., American Fruit Growers, Inc., Hagerstown, Maryland.
Petty, M. A., 120 Old Middletown Road, Pearl River, New York.
Peturson, B., Dominion Laboratory of Plant Pathology, Winnipeg, Manitoba, Canada.
Phillips, Mrs. W. R., 221 Chatsworth Street, Baton Rouge, Louisiana.
Pierce, W. H., Associated Seed Growers, Inc., Hamilton, Montana.
Piersma, S. F., California Packing Co., Rochelle, Illinois.
Pinckard, J. A., Jr., Spring Hill, Alabama.
Pirone, P. P., New York Botanical Gardens, Bronx Park (Fordham P. O.), Bronx, New York 58, New York.
Plakidas, A. G., Botany Department, Louisiana State University, University, Louisiana.
Plunkett, Orda A., Botany Department, University of California at Los Angeles, Los Angeles 24, California.
Poehlman, J. M., Department of Field Crops, University of Missouri, Columbia, Missouri.
Pomerleau, Rene, 241 Parc Champoux, Quebec, P. Q., Canada.
Pon, D. S., Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.
Pontis, Rafael E., Centro Nacional de Investigacion de Café, Federacion Nacional de Cafeteros de Colombia, Chinchina, Caldes, Colombia.
Pontius, Mrs. Hilda C., 4020 Arkansas Avenue, N. W., Washington, D. C.
Pool, Raymond J., University of Nebraska, Lincoln 8, Nebraska.
Poole, R. F., Clemson College, Clemson, South Carolina.
Pope, Seth, Wyeth Laboratories, 111 N. High Street, West Chester, Pennsylvania.
Popp, W., Dominion Rust Research Laboratory, Winnipeg, Manitoba, Canada.
Porte, William S., Plant Industry Station, Beltsville, Maryland.
Porter, C. L., 924 N. Main Street, West Lafayette, Indiana.
*__Porter, Donald R.__, Campbell Soup Co., Box 1406, Sacramento, California.
Porter, Harold L., 2993 Neil Avenue, Columbus 2, Ohio.
Porter, John N., Lederle Laboratories, Inc., Pearl River, New York.
Porter, R. Howard, Edificio Jatobá, Rue Vieira de Carvalho 44 11 Andar, São Paulo, Brazil.
Porter, Richard P., 6315 Gardenia Street, Philadelphia 44, Pennsylvania.
Posey, G. B., 6160 31st Street, N. W., Washington 15, D. C.
Poulos, Panos L., Department of Plant Pathology, Wolf Hall, University of Delaware, Newark, Delaware.
Pound, Glenn S., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.
*__Povah, Alfred H.__, 143 Elmhurst Avenue, Detroit, Michigan.
Powell, Dwight, Illinois Agricultural Experiment Station, Urbana, Illinois.
Prasad, Nirankar, Division of Plant Pathology, University of California, Berkeley, California.
Pratt, Robert M., Department of Plant Pathology, Cornell University, Ithaca, New York.
Presley, John T., Department of Plant Pathology, Agricultural Experiment Station, State College, Mississippi.
Price, W. C., University of Pittsburgh, Pittsburgh 13, Pennsylvania.
Prince, Alton E., Department of Botany, University of Maine, Orono, Maine.
Pryor, Dean E., 3628 E. Colorado Street, East Pasadena, California.
Pu, Mu-Hwa, Peiping State, National Agricultural Research Institute, Peiping, China.
Putnam, Henry N., Box 474, Milwaukee 1, Wisconsin.

Quanjer, H. M., Institut Voor Phytopathologie, Wageningen, Holland.

Racicot, H. N., Division of Botany, Experimental Farm, Ottawa, Ontario, Canada.
Radebaugh, A. D., Blue Mountain Canneries, Dayton, Washington.
Rader, William E., Shell Agricultural Laboratory, Box 1531, Modesto, California.
Raeder, J. M., c/o Department of Plant Pathology, University of Idaho, Moscow, Idaho.
Rainwater, R. Ivan, Box 340, Honolulu, Hawaii.
Raleigh, Walter P., Clyde, Kansas.
Ramsey, G. B., Barnes Laboratory, University of Chicago, 5630 Ingleside Avenue, Chicago 37, Illinois.

Rand, Frederick V., 1359 Meridian Place, N. W., Washington 10, D. C.

Rands, R. D., Plant Industry Station, Beltsville, Maryland.

*__Rankin, W. H.__, Department of Agriculture and Markets, Bureau of Plant Industry, State Office Building, Albany 1, New York.

*__Rawlins, T. E.__, Division of Plant Pathology, University of California, Berkeley 4, California.

Ray, Charles, Jr., California Central Fibre Corp., Pisgah Forest, North Carolina.

Ray, W. W., Department of Botany, University of Nebraska, Lincoln 8, Nebraska.

Raychaudhuri, S. P., Indian Agricultural Research Institute, New Delhi, India.

*__Reddick, Donald__, College of Agriculture, Cornell University, Ithaca, New York.

Reddy, Charles S., Botany Department, Iowa State College, Ames, Iowa.

*__Reed, George M.__, 25 S. Linwood Avenue, Pittsburgh 5, Pennsylvania.

Reed, Howard E., Department of Botany, Ohio State University, Columbus, Ohio.

Reed, T. Walter, California Spray Chemical Corp., Kressan Road, Haddonfield, New Jersey.

Reeves, E. L., Box 99, Wenatchee, Washington.

Reid, David A., Agronomy Department, Kentucky Agricultural Experiment Station, Lexington 29, Kentucky.

Reilly, Joseph J., Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

Reinking, Otto A., New York State Agricultural Experiment Station, Geneva, New York.

Rettew, G. Raymond, West Chester, Pennsylvania.

Rex, E. G., First Mechanics Building, Trenton, New Jersey.

Reycraft, Jean, 661 McKee Avenue, Monessen, Pennsylvania.

Reynard, George B., Campbell Soup Co., Farm No. 1, Research Department, Riverton, New Jersey.

Reynolds, Frank J., 205 West College Avenue, Lafayette, Louisiana.

Reynolds, Harold W., Division of Nematology, Plant Industry Station, Beltsville, Maryland.

*__Rhoads, Arthur S.__, William F. Clapp Laboratory, Inc., Duxbury, Massachusetts.

Rhyne, Claude, Department of Agronomy, State College, Raleigh, North Carolina.

Rice, William N., A. B. M. Agricultural School, Pyinmana, Burma.

Rich, Saul, The Connecticut Agricultural Experiment Station, P.O. Box 1106, New Haven 4, Connecticut.

Richards, B. L., Utah Agricultural College, Logan, Utah.

Richards, B. L., Jr., Pest Control Research Laboratory Experiment Station, E. I. Du Pont Co., Wilmington, Delaware.

Richards, C. A., Forest Products Laboratory, Madison 6, Wisconsin.

Richards, Gerald S., 1910 East 30th Street, Tulsa, Oklahoma.

Richards, M. C., Botany Department, University of New Hampshire, Durham, New Hampshire.

Rieman, G. H., University of Wisconsin, Madison 6, Wisconsin.

*__Riker, A. J.__, Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

Ritchie, C. L., Box 340, Honolulu 9, Hawaii.

Roane, Curtis W., Department of Plant Pathology, Agricultural Experiment Station, Blacksburg, Virginia.

Roane, Mrs. C. W., 108 Eakin St., Blacksburg, Virginia.

Robert, Alice L., Division of Cereal Crops & Diseases, Plant Industry Station, Beltsville, Maryland.

Roberts, Edith A., 17 Dearborn Street, Salem, Massachusetts.

*__Roberts, J. W.__, 116 D Park Avenue S., Winter Park, Florida.

Robles-Gutierrez, Leonel, Instituto Tecnologico y de Estudios Superiores de Monterrey, Monterrey, N. L., Mexico.

Rodenhiser, Herman A., Plant Industry Station, Beltsville, Maryland.

Rodriguez, José, Rafael Rebollar No. 14, Tacubaya, Mexico, D. F.

Rogers, Hoyt, Coker Pedigreed Seed Co., Hartsville, South Carolina.

Rogerson, Clark, Department of Plant Pathology, Cornell University, Ithaca, New York.

Rolfs, F. M., Department of Botany and Plant Pathology, Agricultural and Mechanical College, Stillwater, Oklahoma.

*__Rorer, James B.__, Hacienda San Miguel, Milagro, Ecuador.

Rosberg, David W., Botany Department, Ohio State University, Columbus, Ohio.

Rose, R. C., Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

Rosen, H. R., Department of Plant Pathology, University of Arkansas, Fayetteville, Arkansas.

Ross, A. Frank, Department of Plant Pathology, Cornell University, Ithaca, New York.

Ross, Juan Unzueta, Rio Chico 5, San Angel, Mexico, D. F., Mexico.

Rossetti, Victoria, Instituto Biológico, São Paulo, Brazil.

Roth, Elmer R., 223 Federal Building, Asheville, North Carolina.

Roth, Herbert, 609 West 137th Street, New York 31, New York.

Roth, Lewis F., Department of Botany, Oregon State College, Corvallis, Oregon.

Rowell, John B., Rhode Island Agricultural Experiment Station, Kingston, Rhode Island.

Rudolph, Bert A., 2675 Stevens Creek Road, San Jose, California.

Ruehle, George D., Sub-Tropical Experiment Station, Homestead, Florida.

*__Rumbold, Caroline__, 417 Sterling Place, Madison, Wisconsin.

Runnels, H. A., Ohio Agricultural Experiment Station, Wooster, Ohio.

Rupert, J. A., Box 395, Eveleth, Minnesota.

Ryker, Truman C., Plant Pathology Department, Louisiana State University, Baton Rouge, Louisiana.

Sacco, Paul, 410 Brady Street, Davenport, Iowa.

Sackston, W. E., Dominion Laboratory of Plant Pathology, Winnipeg, Manitoba, Canada.

Saha, Jagadish Chandra, Department of Plant Pathology and Bacteriology, West Virginia University, Morgantown, West Virginia.

Saint John, R. R., DeKalb Hybrid Seed Co., 306 N. Neal Street, Champaign, Illinois.

Sakimura, Kay, Pineapple Research Institute, Honolulu 2, Hawaii.

Samson, R. W., Botany Department, Purdue University, Lafayette, Indiana.

Sands, D. R., Department of Botany, Ontario Agricultural College, Guelph, Ontario, Canada.

Sanford, G. B., Dominion Laboratory of Plant Pathology, University of Alberta, Edmonton, Alberta, Canada.

Santacroce, Nunzio G., 215 Treadwell Avenue, Staten Island 2, New York.

Schafer, John F., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

Schaller, Charles W., Division of Agronomy, University of California, Davis, California.

Schlatter, F. P., 3118 Newton Street, N. E., Washington 18, D. C.

Schlocker, Archie, 321 24th Street, Sacramento, California.

Schmitt, C. G., Boyce Thompson Institute for Plant Research, Yonkers 3, New York.

*__Schmitz, Henry__, College of Agriculture, University Farm, St. Paul 1, Minnesota.

Schneider, Charles L., Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

Schneider, Henry, Division of Plant Pathology, Citrus Experiment Station, Riverside, California.

Schroeder, Wilbur, Division of Plant Pathology, Agricultural Experiment Station, Cornell University, Geneva, New York.

Schuh, Joe, Box 869, Klamath Falls, Oregon.

Schuldt, Paul, Botany Department, Iowa State College, Ames, Iowa.

Schultz, E. S., Plant Industry Station, Beltsville, Maryland.

Schuster, Max L., Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska.

*__Schwarze, C. A.__, 181–20 Midland Parkway, Jamaica, L. I., New York.

Scott, C. Emlen, Division of Plant Pathology, University of California, Berkeley 4, California.

Scott, William W., 176 North Willard, Burlington, Vermont.

Seal, J. L., Auburn, Alabama.

Segall, Raphael, 1232 Sunset Plaza Drive, Los Angeles 46, California.

Seiferle, E. J., 522 Second Street, Belvidere, New Jersey.

Self, Raymond L., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

Seliskar, Carl E., Plant Pathology Department, Cornell University, Ithaca, New York.

Semeniuk, George, Department of Botany, Iowa State College, Ames, Iowa.

Senn, P. H., Agricultural College, Gainesville, Florida.

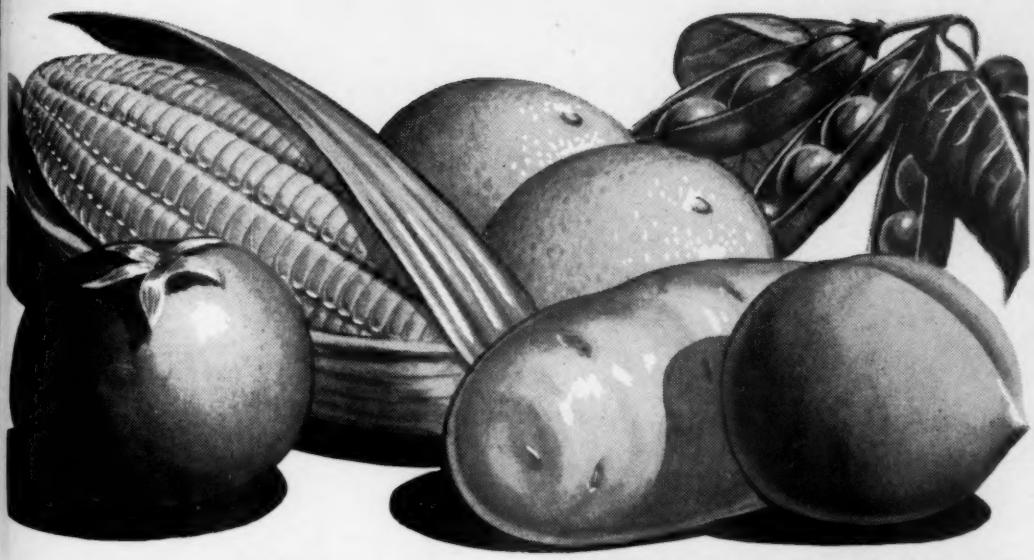
Severin, Henry H. P., Agricultural Hall, University of California, Berkeley 4, California.

Shands, H. L., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

Shands, R. G., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

Shapovalov, Michael, 740 Creek Drive, Menlo Park, California.

Sharvelle, Eric G., Agricultural Experiment Station, Purdue University, Lafayette, Indiana.



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Shaw, Charles G., Plant Pathology Department, Washington State College, Pullman, Washington.

Shaw, K. J., Box 684, Laurinburg, North Carolina.

Shay, J. Ralph, Department of Botany and Plant Pathology, Agricultural Experiment Station, Purdue University, Lafayette, Indiana.

Schafer, Lewis A., Department of Plant Pathology, Kansas State College, Manhattan, Kansas.

*__Shear, C. L.__, 2253 N. Madison Street, Arlington, Virginia.

*__Shear, E. V., Jr.__, Ravena, Albany County, New York.

Shema, Bernard F., 316 E. McKinley Street, Appleton, Wisconsin.

Shepherd, Donald R., 111 Federal Office Building, Minneapolis 1, Minnesota.

Shepherd, H. R., Connecticut Chemical Research Corp., Railroad and Bostwick Avenues, Bridgeport, Connecticut.

Sherbakoff, C. D., Agricultural Experiment Station, University of Tennessee, Knoxville, Tennessee.

Sherf, Arden F., Department of Plant Pathology, College of Agriculture, Lincoln, Nebraska.

Sherwin, Helen, Plant Industry Station, Beltsville, Maryland.

Shippy, W. B., West Side, Sanford, Florida.

Showalter, Joseph W., Scientists Cliffs, Port Republic, Maryland.

Shurtleff, Malcolm C., Jr., Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

*__Sideris, Christos P.__, Pineapple Research Institute, University of Hawaii, Honolulu, Hawaii.

Siggers, Paul V., U. S. Forest Service, Route 1, Saucier, Mississippi.

Silberschmidt, Karl, Instituto Biológico, Caixa Postal 2821, São Paulo, Brazil.

Sill, Webster H., Jr., 234 E. Hamilton Avenue, State College, Pennsylvania.

Silveira, Verlande Duarte, Rua Visconde de Pirajá 583c. 1, Rio de Janeiro, Brazil.

Silverborg, Savel B., New York State College of Forestry, Syracuse University, Syracuse, New York.

Simmonds, P. M., Dominion Laboratory of Plant Pathology, University of Saskatchewan, Saskatoon, Sask., Canada.

Simmons, S. A., Department of Botany, Ontario Agricultural College, Guelph, Ontario, Canada.

Simpson, Dwight M., U. S. Cotton Field Station, Knoxville, Tennessee.

Simpson, G. W., Holmes Hall, Orono, Maine.

Sinden, James, Department of Botany, Pennsylvania State College, State College, Pennsylvania.

Sisson, Max A., Box 145, Palisade, Colorado.

Silva, Ady Raul da, Estação Experimental de Trigo, Curitiba, Paraná, Brazil.

Slade, M. A., Jr., Food Machinery Corp., Lakeland, Florida.

**__Slagg, C. M.__, Department of Botany and Plant Pathology, Kansas State College, Manhattan, Kansas.

Sleeth, Bailey, 1461 7th Avenue, Yuma, Arizona.

Slipp, A. W., School of Forestry, University of Idaho, Moscow, Idaho.

Slutz, Russell C., High Street, Navarre, Ohio.

*__Small, Cyril G.__, 81 West Main Street, Sodus, New York.

Smith, A. L., Department of Agronomy, Alabama Experiment Station, Auburn, Alabama.

Smith, Charles L., 333 Graham Street, Highland Park, New Jersey.

Smith, Clayton O., Citrus Experiment Station, Riverside, California.

Smith, Donald J., 1023 S. E. Saint Charles Street, Brownsville, Texas.

Smith, Edward, 127 U. S. Court House, El Paso, Texas.

Smith, Floyd F., Plant Industry Station, Beltsville, Maryland.

*__Smith, George H.__, 1925 Central Avenue, Indianapolis 2, Indiana.

Smith, Kenneth M., Plant Virus Research Unit, Molteno Institute, Cambridge, England.

Smith, M. A., U. S. Department of Agriculture, 5630 Ingleside Avenue, Chicago 37, Illinois.

Smith, Oliver F., Agricultural Experiment Station, University of Nevada, Reno, Nevada.

Smith, Paul G., Division of Truck Crops, University of California, Davis, California.

Smith, Thomas E., McNair's Yield-Tested Seed Co., Box 427, Laurinburg, North Carolina.

Smith, Wilson L., Jr., Plant Pathology Department, Cornell University, Ithaca, New York.

Smoot, John J., Botany Department, University of Maryland, College Park, Maryland.

Smucker, S. J., Rensselaer, Indiana.

*__Snell, Walter H.__, Brown University, Providence 12, Rhode Island.

Snieszko, S. F., U. S. Fisheries Station, Kearneysville, West Virginia.

Snyder, William C., Department of Plant Pathology, University of California, Berkeley 4, California.

Solheim, Wilhelm G., Botany Department, University of Wyoming, Laramie, Wyoming.

Sorrell, Morton B., 35–54 73rd Street, Jackson Heights, New York.

Souffant, Rafael H., Box 8027, Louisiana State University, Baton Rouge, Louisiana.

Spasoff, Luben, Tobacco Research Laboratory, Chatham, Virginia.

Spaulding, Perley, 360 Prospect Street, New Haven 11, Connecticut.

Spears, Richard K., Jr., Department of Botany, Louisiana State University, Baton Rouge 3, Louisiana.

Spencer, E. R., Lebanon, Illinois.

Speroni, Horacio, Melo 2472, Buenos Aires, Argentina.

Spiegelberg, Carl H., P. R. 1, Box 3166, Honolulu 2, Hawaii.

*__Sprague, Roderick__, Department of Plant Pathology, Washington State College, Pullman, Washington.

Sproston, Thomas, Jr., Science Hall, University of Vermont, Burlington, Vermont.

Stahel, Gerold, Agricultural Experiment Station, Paramaribo, Surinam, Dutch Guiana.

*__Stakman, E. C.__, Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.

Standen, J. H., 373 Park Ave., Yonkers 3, New York.

Stanley, Alfred R., Commercial Solvents Corporation, Terre Haute, Indiana.

Stanley, Wendell M., Department of Biochemistry, University of California, Berkeley 4, California.

Stanton, T. Ray, Plant Industry Station, Beltsville, Maryland.

Stark, F. L., Jr., American Cyanamid Co., Stamford, Connecticut.

Starr, G. H., Department of Plant Pathology, University of Wyoming, Laramie, Wyoming.

Starr, Mortimer P., Division of Bacteriology, University of California, Davis, California.

Staten, Glen, Agricultural Experiment Station, State College, New Mexico.

Steenland, Adin P., Oregon Experiment Station, Oregon State College, Corvallis, Oregon.

Steere, Russell L., Botany Department, University of Michigan, Ann Arbor, Michigan.

Steib, Rene J., Botany Department, Louisiana State University, Baton Rouge, Louisiana.

Steiner, G., Plant Industry Station, Beltsville, Maryland.

Steinmetz, F. H., 36 College Avenue, Orono, Maine.

Stevens, H. E., Amherst Apartments, Orlando, Florida.

*__Stevens, Neil E.__, Department of Botany, University of Illinois, Urbana, Illinois.

Stevens, Russell B., Department of Botany, University of Tennessee, Knoxville, Tennessee.

Stevenson, E. C., Department of Horticulture, Purdue University, Lafayette, Indiana.

Stevenson, Frank V., California Polytechnic College, San Luis Obispo, California.

Stevenson, Frederick J., 508 Ethan Allen Avenue, Tacoma Park, Maryland.

Stevenson, John A., 4113 Emery Place, Washington 16, D. C.

Stevenson, M. B., Jr., Clemson College, Clemson, South Carolina.

Stewart, Dewey, 1736 Webster Street, N. E., Washington 17, D. C.

Stewart, Donald M., 309 Federal Building, Duluth 2, Minnesota.

Steyaert, Rene L., 15g Rue des ATREBATES, Etterbeek, Bruxelles IV, Belgium.

Stillinger, C. R., 418 College Avenue, Moscow, Idaho.

Stirland, L. L., 4026 Du Pont Building, Wilmington, Delaware.

Stoddard, David L., Everglades Experiment Station, Box 37, Belle Glade, Florida.

Stoddard, E. M., Connecticut Agricultural Experiment Station, New Haven 4, Connecticut.

Stoller, B. B., Lennys Foods Inc., 525 Lake Avenue S., Duluth, Minnesota.

Stone, William E., Laboratorio Entomologico, Apartado Postal 3, Colonia Anahuac, D. F., Mexico.

Stoner, Warren N., 2520 Ridge Road, Berkeley, California.

*__Storey, H. H.__, Colonial Office, London S. W., England.

Stout, Gilbert L., Bureau of Plant Pathology, State Department of Agriculture, Sacramento 14, California.

*__Stover, W. C.__, Department of Botany, Ohio State University, Columbus 10, Ohio.

Strong, Forrest, 1213 N. Walnut Street, Lansing, Michigan.

Strong, Miriam C., 1213 N. Walnut Street, Lansing 6, Michigan.

Struble, F. Ben, Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

Stutzman, Roy, Prescott, Wisconsin.
Suit, R. F., Citrus Experiment Station, Lake Alfred, Florida.
Summers, Thomas E., Iowa State College, Ames, Iowa.
Swank, George, Jr., 186 W. Woodruff Avenue, Columbus 1, Ohio.
Swartwout, H. G., Department of Horticulture and Forestry, University of Missouri, Columbia, Missouri.
Swingle, Roger U., Box 244, Station G., Columbus, Ohio.
Sylvester, E. S., 112 Agricultural Hall, University of California, Berkeley 4, California.
Szkolnik, Michael, Agricultural Experiment Station, New Brunswick, New Jersey.

Takahashi, William N., 107 Hilgard Hall, University of California, Berkeley 4, California.
Tapke, Victor F., Plant Industry Station, Beltsville, Maryland.
Tarjan, Armen C., Department of Botany, University of Maryland, College Park, Maryland.
Tartakowsky, Sergio, Box 4647, Santiago, Chile.
Tasche, Carl H., 24500 Walnut Street, Lomita, California.
Taylor, Albert L., 1415 Liberty Life Building, Shell Chemical Corp., Charlotte 2, North Carolina.
Taylor, C. F., Department of Plant Pathology and Bacteriology, West Virginia University, Morgantown, West Virginia.
Tehon, Leo R., 337 Natural Resources Building, Urbana, Illinois.
Teller, Morris N., Division of Plant Pathology, University Farm, St. Paul 1, Minnesota.
Templeton, Bonnie C., Los Angeles Museum, Exposition Park, Los Angeles 7, California.
Tervet, Ian, Camp Detrick, Frederick, Maryland.
Texera, Diego A., Instituto de Investigaciones Veterinarias, El Valle, Caracas, Venezuela.
Thanos, Andrew, Department of Plant Pathology and Bacteriology, West Virginia University, Morgantown, West Virginia.
Theis, Thomas N., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.
Thind, Kartar Singh, Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.
Thirumalachar, M. J., 20th 5th Road, Malleswaram, Bangalore, India.
Thomas, Charles A., Tobacco, Medicinal and Special Crops, Plant Industry Station, Beltsville, Maryland.
Thomas, Harold E., Route 2, Box 219, Morgan Hill, California.
Thomas, H. Earl, Division of Plant Pathology, University of California, Berkeley 4, California.
Thomas, H. Rex, Division of Fruit and Vegetable Crops and Diseases, Plant Industry Station, Beltsville, Maryland.
Thomas, John E., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.
Thomas, Roy C., Ohio Agricultural Experiment Station, Wooster, Ohio.
Thomas, Samuel O., 802 Bancroft Hall, 509 W. 121st Street, New York 27, New York.
Thomas, W. D., Jr., Department of Botany and Plant Pathology, Colorado State College, Fort Collins, Colorado.
Thompson, A. Robert, 958 Spruce Street, Winnetka, Illinois.
Thompson, G. E., Department of Plant Pathology, College of Agriculture, University of Georgia, Athens, Georgia.
*__Thornberry, H. H.__, Department of Horticulture, University of Illinois, Urbana, Illinois.
Thornton, Norwood C., Carbide and Carbon Chemical Corp., 30 E. 42nd Street, New York 17, New York.
Thung, T. H., J. P. Coenweg 8, Buitenzorg, Java, East Indies.
Thurston, H. W., Pennsylvania State College, State College, Pennsylvania.
Tidd, Joseph S., Associated Seed Growers, New Haven, Connecticut.
Tilford, Paul E., Box 427, Wooster, Ohio.
Tims, Eugene C., Agricultural Experiment Station, University, Louisiana.
Tint, Howard, 332 East Olney Avenue, Philadelphia 20, Pennsylvania.
*__Tisdale, W. B.__, Agricultural Experiment Station, Gainesville, Florida.
Tisdale, W. H., Du Pont Experiment Station, Wilmington 98, Delaware.
Todd, Furney A., Botany Department, North Carolina State College, Raleigh, North Carolina.
Tolaas, A. G., University Farm, St. Paul 1, Minnesota.
*__Tompkins, C. M.__, Division of Plant Pathology, University of California, Berkeley 4, California.
Topping, Elizabeth R., Ventura County Library, Box 771, Ventura, California.
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Trelease, Sam F., Department of Botany, Columbia University, New York 27, New York.

True, R. P., Southern Forest Experiment Station, Box 92, Lake City, Florida.

****Tu, Chih**, Department of Reconstruction, Tihwa, Sinkiang, China.

Tucker, C. M., Department of Botany, University of Missouri, Columbia, Missouri.

Tullis, E. C., Box 2967, Beaumont, Texas.

Tyler, Leon J., Department of Plant Pathology, Cornell University, Ithaca, New York.

Tyner, L. E., Dominion Laboratory of Plant Pathology, University of Alberta, Edmonton, Alberta, Canada.

Ukkelberg, Harry G., Richmond Hill, Georgia.

Ullstrup, Arnold J., Department of Botany, Agricultural Experiment Station, Lafayette, Indiana.

Urries, Manuel de, Jardín Botánico, Plaza de Murillo 2, Madrid, Spain.

Utech, Nedra M., 2 Horticulture Building, University of Wisconsin, Madison 6, Wisconsin.

Utter, L. Gordon, Phelps Dodge Refining Corp., 40 Wall Street, New York 5, New York.

Valleau, W. D., Kentucky Agricultural Experiment Station, Lexington 29, Kentucky.

Vallega, José, Beauchef 411, Departamento A, Buenos Aires, Argentina.

Van Burgh, Patricia, Botany Department, Ohio State University, Columbus, Ohio.

Van Dillweijn, C., Ataturk Bulvari 327, Ankara, Turkey.

Van Gelowe, J. D., G. L. F. Building, Terrace Hill, Ithaca, New York.

Van Meter, Mary G., 1316 New Hampshire Avenue, N. W., Washington 6, D. C.

Van Schaack, Valerie, University of Arizona, Tucson, Arizona.

Van Slogteren, E., Laboratorium voor Bloem, Lisse, Holland.

Vanterpool, T. C., Department of Biology, University of Saskatchewan, Saskatoon, Sask., Canada.

Vasudeva, R. S., Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, India.

Vaughan, Edward K., Botany Department, Oregon State College, Corvallis, Oregon.

Vaughan, Leonard H., Vaughan's Seed Store, 601–609 West Jackson Boulevard, Chicago 6, Illinois.

***Vaughan, R. E.**, Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

Vaughn, J. R., Michigan State College, Department of Botany and Plant Pathology, East Lansing, Michigan.

Vermillion, M. T., Earich Road, Route 3, Athens, Ohio.

Vestal, Edgar F., Agricultural Institute, Allahabad, U. P., India.

Virgin, Walter J., California Packing Corp., Route 2, Box 309B, Mountain View, California.

Vlitos, August John, 232 Duck Street, Stillwater, Oklahoma.

Von Schrenk, Hermann, Tower Grove & Flad Avenue, St. Louis, Missouri.

Voorhees, R. K., Box 232, Fort Pierce, Florida.

Wadley, Bryce N., Botany Department, Utah State Agricultural College, Logan, Utah.

Wagener, Willis W., 720 Appraisers Building, 630 Sansome Street, San Francisco 11, California.

Wahl, Izaak, Department of Plant Pathology and Botany, University Farm, St. Paul 1, Minnesota.

Waldee, E. L., Department of Botany and Plant Pathology, Utah State Agricultural College, Logan, Utah.

Walker, A. R., The University of Western Ontario, London, Ontario, Canada.

Walker, Earnest A., 6903 Carleton Terrace, College Park, Maryland.

***Walker, J. C.**, Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

Walker, Marion N., 1761 Alisal Road, Salinas, California.

Wallace, Errett, 3806 Hamilton Street, Hyattsville, Maryland.

Wallace, H. A. H., Dominion Rust Laboratory, University of Manitoba, Winnipeg, Manitoba, Canada.

Wallace, J. M., Citrus Experiment Station, University of California, Riverside, California.

Wallace, Philip P., 659 North Clay Avenue, Kirkwood, Missouri.

Wallin, Jack R., Botany Department, Iowa State College, Ames, Iowa.

Walter, James M., Vegetable Crops Laboratory, Bradenton, Florida.

Walters, Hubert J., Plant Pathology Department, University of Nebraska, Lincoln 1, Nebraska.

Wang, Chuang, 139 Kweinan Road, Kweilin, Kwangsi, China.

Wang, C. S., College of Agriculture, Honan University, Kaifeng, Honan, China.

Wang, Huan-Ru, Department of Plant Pathology, Peking University, Peiping, China.

Wang, T. M., Department of Plant Pathology, University of Nanking, Nanking, China.

Warner, Robert M., California Fig Institute Research Laboratory, 7105 Palm Ave., Fresno, California.

Warren, John R., Botany Department, Duke University, Durham, North Carolina.

Waterhouse, W. L., Faculty of Agriculture, The University of Sydney, Sydney, Australia.

Waterman, Alma M., 360 Prospect Street, New Haven 11, Connecticut.

Watkins, G. M., 10103 Mc Kinney Ave., Silver Spring, Maryland.

Watson, Ross D., Department of Plant Pathology, University of Idaho, Moscow, Idaho.

Watson, William M., Box 831, Bluefield, West Virginia.

Weaver, Leroy E., 56 Marion Street, Salinas, California.

Weaver, L. O., Plant Pathology Division, University of Maryland, College Park, Maryland.

*__Weber, George F.,__ Agricultural Experiment Station, Gainesville, Florida.

Weber, Paul V. V., 305 Lathrop Street, Madison 5, Wisconsin.

Webster, O. J., College of Agriculture, Lincoln, Nebraska.

Weetman, Leslie M., U. S. Sugar Corp., Clewiston, Florida.

Wei, C. T., Department of Plant Pathology, University of Nanking, Nanking, China.

Weimer, J. L., Agricultural Experiment Station, Experiment, Georgia.

Weindling, R., Lederle Laboratories, Pearl River, New York.

Weiss, Freeman, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

Welch, A. W., Du Pont Semesan Laboratory, 101 West 10th Street, Wilmington 98, Delaware.

Welch, D. S., Department of Plant Pathology, Cornell University, Ithaca, New York.

Wellhausen, Edwin J., Calle Viena 26, Mexico, D.F., Mexico.

Wellman, F. L., Office of Foreign Agricultural Relations, U. S. Department of Agriculture, Washington 25, D. C.

Wellman, Richard H., Boyce Thompson Institute, Yonkers 3, New York.

Wernham, C. C., Department of Botany, State College, Pennsylvania.

*__West, Erdman,__ Agricultural Experiment Station, Gainesville, Florida.

West, Floyd R., 1090 P. A. W., Mansfield, Ohio.

West, Harry, 2 South Hartland Street, Middleport, New York.

Westcott, Cynthia, 96 Essex Avenue, Glen Ridge, New Jersey.

Wester, Horace V., National Capital Parks, Department of Interior, Washington, D. C.

*__Weston, William H.,__ Biological Laboratories D 482, Harvard University, Cambridge, Massachusetts.

Wheeler, Harry E., 205 East State Street, Baton Rouge, Louisiana.

Wheeler, Willis H., 3800 18th Street N., Arlington, Virginia.

Whitaker, Thomas W., Box 150, La Jolla, California.

White, Alec, 705 Madison Street, Tampa 2, Florida.

*__White, Richard P.,__ 5367 28th Street, N. W., Washington 15, D. C.

*__Whitney, Will A.,__ c/o Jay Emanuel Publications Inc., 1225 Vine Street, Philadelphia 7, Pennsylvania.

Wiant, J. S., Room 1022, 641 Washington Street, New York 14, New York.

Wieman, J. S., 805 Terminal Sales Building, Portland 5, Oregon.

Wilcox, Marguerite S., Plant Industry Station, Beltsville, Maryland.

Wilcox, Raymond B., U. S. Department of Agriculture, Pemberton, New Jersey.

Wilcoxon, Frank, 142 Hollow Tree, Ridge Road, Noroton Heights, Connecticut.

Wilds, George J., Coker's Pedigreed Seed Co., Hartsville, South Carolina.

Wilhelm, Stephen, Division of Plant Pathology, College of Agriculture, University of California, Berkeley 4, California.

Wilkinson, Robert, Plant Pathology Department, Cornell University, Ithaca, New York.

Williams, Harold E., 1111 Bennett Avenue, Prosser, Washington.

Williamson, Charles E., Department of Plant Pathology, Cornell University, Ithaca, New York.

Willison, R. S., Box 87, St. Catharines, Ontario, Canada.

*__Wilson, A. R.,__ The Midland Agricultural College, Sutton Bonington, Loughborough, Leicestershire, England.

Wilson, Coyt, Department of Botany and Plant Pathology, Alabama Polytechnic Institute, Auburn, Alabama.

Wilson, E. E., Division of Plant Pathology, University Farm, Davis, California.

Wilson, Elmer L., Botany Hall, Iowa State College, Ames, Iowa.

Wilson, J. Dean, Ohio Agricultural Experiment Station, Wooster, Ohio.

Wilson, O. T., Department of Botany, University of Cincinnati, Cincinnati 21, Ohio.

Wilson, W. E., Department of Botany, Miami University, Oxford, Ohio.

Wingard, S. A., Department of Botany and Plant Pathology, Agricultural Experiment Station, Blacksburg, Virginia.

Winston, J. R., 415 N. Parramore Street, Orlando, Florida.

Winter, Floyd L., c/o Associated Seed Growers Inc., New Haven 2, Connecticut.

Winter, H. F., Ohio Agricultural Experiment Station, Wooster, Ohio.

Winterhead, Marvin D., Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

Wischhusen, J. F., Manganese Research and Development Foundation, 15031 Shore Acres Drive, Cleveland 10, Ohio.

Wismer, Chester A., H. S. P. A. Experiment Station, Honolulu, Hawaii.

Wolcott, Lawrence, 23 Castle Heights Avenue, Nyack, New York.

*__Wolf, Frederick A.__, Duke University, Durham, North Carolina.

Wolfe, Raymond F., 3211 Indianola Avenue, Columbus, Ohio.

Wood, F. C., The Rest, Franklin Road, Durrington, Worthing, England.

Wood, William B., Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Washington 25, D. C.

Woodroof, Mrs. N. C., Coastal Plain Experimental Station, Tifton, Georgia.

Woods, Mark, 4003 Quintana Street, Hyattsville, Maryland.

Worthley, H. N., Merck & Co., Rahway, New Jersey.

Wright, Charles M., R.F.D. 1, Pullman, Washington.

*__Wright, Ernest__, Division of Forest Pathology, Box 4137, Portland 8, Oregon.

Wright, L. K., Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Washington 25, D. C.

Wright, Theodore R., Box 344, Wenatchee, Washington.

Wynd, F. L., Department of Botany and Plant Pathology, Michigan State College, East Lansing, Michigan.

Wysong, Noel B., 536 N. Harlem Avenue, River Forest, Illinois.

Yarwood, Cecil, Division of Plant Pathology, University of California, Berkeley 4, California.

Yoder, Donald M., 414 Stewart Avenue, Ithaca, New York.

Yost, Henry E., 849 Collicello Street, Harrisonburg, Virginia.

Young, H. C., Department of Botany, Ohio Agricultural Experiment Station, Wooster, Ohio.

Young, Harry C., Jr., Division of Plant Pathology, New York Agricultural Experiment Station, Geneva, New York.

*__Young, Paul A.__, R. R. 3, Jacksonville, Texas.

Young, Roy A., Botany Department, Iowa State College, Ames, Iowa.

Young, V. H., Department of Plant Pathology, University of Arkansas, Fayetteville, Arkansas.

Younkin, S. G., Research Department, Campbell Soup Co., Riverton, New Jersey.

Yount, Marion E., Barberry Eradication Office, College of Agriculture, Lincoln 1, Nebraska.

Yount, W. L., 619 Cascadella Street, Ithaca, New York.

Yu, T. F., College of Agriculture, The National University of Peking, Peiping, China.

Zabel, Robert A., 951 Ackerman Avenue, Syracuse 10, New York.

Zaumeyer, William J., Plant Industry Station, Beltsville, Maryland.

Zeller, Sanford M., Department of Botany, Oregon State College, Corvallis, Oregon.

Zender, Justin, Schenley Laboratories Inc., Lawrenceburg, Indiana.

Zenteno S., Martha, Oficina de Estudios Especiales, Dirección General de Agricultura, San Jacinto, D.F., Mexico.

Zentmyer, George A., Citrus Experiment Station, Riverside, California.

Ziffer, Jack, Research Department, A. E. Staley Manufacturing Co., Decatur, Illinois.

Zorie, Joseph G., Veterans Trailer Camp, Colorado State College, Fort Collins, Colorado.

Zuck, Robert K., Drew University, Madison, New Jersey.

Zuckerman, H. G., 501 Bank of America Building, Berkeley 4, California.

Zundel, George L., 202 Agricultural Education Building, Pennsylvania State College, State College, Pennsylvania.

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	Elected	Died
G. W. Goldsmith	1938	1943
Ernest Adam Dopp	1930	1944
Edward Franklin Gaines	1924	1944
Herbert Hice Whetzel (charter)	1911	1944
James Peter Jolivette	1941	1945
Albert E. Edgecombe	1930	1945
Lewis Ralph Jones (charter)	1911	1945
Merton Benway Waite (charter)	1911	1945
Leon Hatchig Leonian (life)	1925	1945
Chalmers Jackson King	1923	1945
Rodney Beecher Harvey	1921	1945
John L. Rue	1941	1946
Grover H. Burnett (life)	1929	1946
R. A. Harper (charter)	1911	1946
George Grant Hedgcock (charter)	1911	1946
G. A. Scott	1943	1946
Fred Carleton Stewart (charter)	1911	1946
A. E. Davey	1930	1947
Albert Isaksson	1945	1947
Ralph Justo-Prats	1941	1947
Joseph Hunter Gooding, Jr.	1923	1947
William Moore Scott (charter, life)	charter	1947
H. Loran Blood	1911	1948
Anthony Berg	1916	1948
Cyril O. Bratley (life)	1926	1948
Albert F. Woods (charter, life)	1911	1948

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